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# RESISTANCE OF SEEDS TO DESICCATION

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## INTRODUCTION

The question of the resistance of seeds to extreme drying probably first came into prominence as a result of the use of artificial desiccation to hasten afterripening, as reported by Hotter, Nobbe, Atterberg, Kiessling, and others. The drying was usually done in drying ovens, and the temperatures used frequently were not such as to reduce the moisture content of the seeds below 5 or 6 per cent. Sometimes, however, when higher temperatures were used, the moisture content fell considerably lower than this, and in such cases the germination was often lowered. The question, of course, arises whether the injury comes from the loss of moisture or as the effect of the high temperatures used.

## HISTORICAL REVIEW

Schröeder [1] dried barley and wheat for 12 weeks over sulphuric acid. At the end of this time they contained only 1 to 2 per cent of moisture, but nearly all germinated. Nobbe [2] reduced the water content of rye to 1.2 per cent by drying it at 80° C. with very little effect upon subsequent germination. More severe drying by heat seriously injured the germination of rye, and less severe drying had an injurious effect upon wheat and oats. About the same time Ewart,[3] working with seeds of wheat, corn, barley, peas, haricots, hemp, squash, rape, and sunflower, which he dried in a vacuum desiccator at 37 to 38° C., concluded that it is impossible to reduce the percentage of water held by even the most resistant seeds to lower than 2 or 3 per cent of their dry weight without injuriously affecting their vitality. Ewart's hypothesis was that excessive drying so changed the dormant protoplasm that upon being remoistened it was unable to reestablish the molecular groupings essential for normal vital activity.

[1] SCHRÖDER, G. ÜBER DIE AUSTROCKNUNGSFÄHIGKEIT DER PFLANZEN. *In* Untersuch. Bot. Inst. Tübingen, Bd. 2, Heft 1, p. 1–52. 1886.

[2] NOBBE, F. ÜBER KÜNSTLICHE GETREIDETROCKNUNG MIT BEZUG AUF DIE KEIMFÄHIGKEIT. *In* Mitt. Deut. Landw. Gesell., Jahrg. 12, Stück 14, p. 185–186. 1897.

[3] EWART, A. J. ADDITIONAL OBSERVATIONS ON THE VITALITY AND GERMINATION OF SEEDS. *In* Proc. and Trans. Liverpool Biol. Soc., v. 10, 1895/96, p. 185–193, 5 pl. 1896.

Since Ewart published his papers, other investigators have put out results which seem to controvert his conclusions. Pickholz,[1] by storing over sulphuric-acid solutions and by drying over concentrated sulphuric acid *in vacuo*, varied the moisture content of Kentucky bluegrass seed from a mere trace to 32 per cent. The lower the moisture content at the time of beginning the germination test, the more rapid and complete was the germination at 20° C., though germination was little affected at 28°, or with a daily alternation between 20 and 28°. Seeds with a mere trace of water present germinated almost as well at the usually very unfavorable temperature 20°, as with the favorable alternation between 20° and 28°. Waggoner,[2] by drying first at 60° and later at 100° C., reduced the moisture content of radish seeds to 0.4 per cent without affecting subsequent germination.

## EXPERIMENTAL WORK

The work of the present authors with seeds of a number of species of Gramineae corroborates the results of Pickholz and Waggoner. It should be noted, however, that some kinds of seeds are known to be unable to withstand even ordinary air-drying. Among these are the seeds of silver maple (*Acer saccharinum*), wild rice (*Zizania palustris*), the various species of willows (*Salix* spp.), and many water plants.

In the winter of 1913 seeds of Kentucky bluegrass (*Poa pratensis* L.) of 90 per cent germinating capacity were dried *in vacuo* over calcium oxid (CaO) at room temperature. With 1.5 per cent of moisture remaining in the seeds there was no fall in germinating capacity or in germinating energy. With 0.2 per cent moisture the germinating capacity remained the same, but the germinating energy was considerably less. With 0.1 per cent moisture the germinating capacity had fallen about 5 per cent, and the germinating energy was seriously reduced. One lot previously dried in a lime desiccator to 0.1 per cent of moisture was further dried in a vacuum oven at 100° C. for six hours to remove the last trace of moisture. The germinating energy (see below) was thus reduced to one-half what it was with 0.1 per cent of moisture, and the seedlings produced were weak; but the percentage of seeds which germinated remained the same as before removing the last 0.1 per cent of the water. All of the germination tests were conducted in the Jacobson apparatus with a daily alternation of temperatures between 20° and 30° C. All of the tests with a quick-germinating lot were continued for 28 days, and the ratio between the percentage of germination at the end

[1] PICKHOLZ, L. EIN BEITRAG ZUR FRAGE ÜBER DIE WIRKUNG DES LICHTES UND DER INTERMITTIERENDEN TEMPERATURE AUF DIE KEIMUNG VON SAMEN, SOWIE ÜBER DIE ROLLE DES WASSERGEHALTES DER SAMEN BEI DIESER WIRKUNG. *In* Ztschr. Landw. Versuchsw. Oesterr., Bd. 14, Heft 2, p. 124–151, 1 fig. 1911. Literaturverzeichnis, p. 150–151.

[2] WAGGONER, H. D. THE VIABILITY OF RADISH SEEDS (RAPHANUS SATIVUS L.) AS AFFECTED BY HIGH TEMPERATURES AND WATER CONTENT. *In* Amer. Jour. Bot., v. 4, no. 5, p. 299–323, 1 fig. 1917. Literature, p. 312–313.

of 7 days and that at the end of 28 days was used as a measure of the germinating energy. With a lot of seed which germinated more slowly all germination tests were continued for 35 days, and the ratio 14 to 35 days was used to express the germinating energy.

The series of experiments described in the following pages was begun on January 12, 1917. Two varieties of barley, *Hordeum vulgare* L.; two varieties of wheat, *Triticum aestivum* L. (*T. vulgare* Vill.); a sample of Sudan grass, *Holcus halepensis sudanensis* (Piper) Hitchcock (*Andropogon halepensis sudanensis* Piper); and one of Johnson grass, *Holcus halepensis* L. (*Sorghum halepense* Pers.) were stored at room temperature in evacuated desiccators over calcium oxid and over concentrated sulphuric acid. From the seeds in these desiccators and from control lots stored in an open vessel in a desk drawer samples were withdrawn for moisture determinations and germination tests at intervals during the next 10½ months. Germination tests of Johnson grass seed were made with a daily alternation of temperatures between 25° and 40° C. All tests were continued for from 9 to 17 days. Germination tests of all other seeds were made at 20° C., and the length of the tests varied from four to six days, germination being practically completed in four days. In all germination tests moist blotting-paper disks inclosed in 100-mm. petri dishes were used for germinating beds. The results of moisture determinations and germination tests are summarized in Tables I, II, and III.

TABLE I.—*Moisture content and percentage of germination of Svanhals barley and Johnson grass samples after storage for different lengths of time over lime and over sulphuric acid, compared with samples stored in an open vessel*

| Date of test. | Length of time stored under different conditions. | Svanhals barley. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moisture content (percentage of dry weight). | | | Germination (per cent). | | |
| | | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. |
| 1917. | | | | | | | |
| Jan. 12 | ..... | ..... | ..... | 9.1 | ..... | ..... | 99 |
| Feb. 26 | 6 weeks..... | 3.4 | 4.1 | ..... | 97 | 96 | 95 |
| Apr. 2 | 11 weeks..... | 2.7 | 2.9 | ..... | 96 | 99 | ..... |
| May 3 | 16 weeks..... | 1.5 | 1.8 | 8.2 | 97 | 100 | 99 |
| June 19 | 23 weeks..... | 1.7 | 1.9 | 11.8 | 95 | 94 | 98 |
| July 12 | 6 months..... | 1.1 | 1.3 | 12.1 | 96 | 98 | 98 |
| Aug. 8 | 7 months..... | .7 | .9 | ..... | 95 | 98 | 98 |
| Sept. 5 | 8 months..... | ..... | ..... | ..... | 97 | 97 | 97 |
| Nov. 25 | 10½ months..... | .8 | .7 | 9.4 | 98 | 96 | 96 |

TABLE I.—*Moisture content and percentage of germination of Svanhals barley and Johnson grass samples after storage for different lengths of time over lime and over sulphuric acid, compared with samples stored in an open vessel*—Continued

| Date of test. | Length of time stored under different conditions. | Johnson grass. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moisture content (percentage of dry weight). | | | Germination (per cent). | | |
| | | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. |
| 1917. | | | | | | | |
| Feb. 26 | 6 weeks | 3.3 | 3.2 | ..... | ..... | ..... | ..... |
| Apr. 2 | 11 weeks | 1.8 | 2.1 | ..... | 58 | 42 | 65 |
| May 3 | 16 weeks | 1.3 | 1.6 | 8.4 | 64 | 64 | 66 |
| June 19 | 23 weeks | 1.2 | 1.6 | 11.7 | 47 | 58 | 58 |
| July 12 | 6 months | .6 | .8 | 11.5 | 65 | 56 | 56 |
| Aug. 8 | 7 months | .4 | .5 | ..... | 48 | 48 | 68 |
| Sept. 5 | 8 months | ..... | ..... | ..... | 52 | 60 | 78 |
| Nov. 25 | 10½ months | .1 | .2 | 9.3 | 80 | 78 | 82 |

TABLE II.—*Moisture content and percentages of germination of White Smyrna barley and Sudan grass samples after storage for different lengths of time over lime and over sulphuric acid, compared with samples stored in an open vessel*

| Date of test. | Length of time stored under different conditions. | White Smyrna barley. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moisture content (percentage of dry weight). | | | Germination (per cent). | | |
| | | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. |
| 1917. | *Months.* | | | | | | |
| Jan. 12 | ..... | ..... | ..... | 9.3 | ..... | ..... | 87 |
| July 12 | 6 | 1.1 | 1.3 | 12.2 | 88 | 88 | 92 |
| Aug. 8 | 7 | .7 | .7 | ..... | 87 | 90 | 91 |
| Sept. 5 | 8 | ..... | ..... | ..... | 84 | 88 | 86 |
| Nov. 25 | 10½ | .6 | .6 | 9.5 | 85 | 86 | 82 |

| Date of test. | Length of time stored under different conditions. | Sudan grass. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moisture content (percentage of dry weight). | | | Germination (per cent). | | |
| | | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. |
| 1917. | *Months.* | | | | | | |
| Jan. 12 | ..... | ..... | ..... | ..... | ..... | ..... | 90 |
| July 12 | 6 | 0.9 | 1.2 | 12.1 | 92 | 94 | 92 |
| Aug. 8 | 7 | .6 | ..... | ..... | 81 | 94 | 94 |
| Sept. 5 | 8 | ..... | ..... | ..... | 86 | 91 | 92 |
| Nov. 25 | 10½ | .6 | .5 | 9.7 | 90 | 92 | 95 |

TABLE III.—*Moisture content and percentages of germination of samples of wheat after storage for different lengths of time over lime and over sulphuric acid, compared with samples stored in an open vessel*

| Date of test. | Length of time stored under different conditions. | Kharkof wheat. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moisture content (percentage of dry weight). | | | Germination (per cent). | | |
| | | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. |
| 1917. | *Months.* | | | | | | |
| Jan. 12 | | ..... | ..... | 9.4 | ..... | ..... | 89 |
| July 12 | 6 | 1.5 | 1.7 | 12.7 | 83 | 84 | 90 |
| Aug. 8 | 7 | .9 | 1.1 | ..... | 87 | 90 | 93 |

| Date of test. | Length of time stored under different conditions. | Pelissier wheat. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moisture content (percentage of dry weight). | | | Germination (per cent). | | |
| | | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. | Stored over calcium oxid. | Stored over sulphuric acid (sp. gr. 1.84). | Stored in open vessel. |
| 1917. | *Months.* | | | | | | |
| Jan. 12 | | ..... | ..... | 9.2 | ..... | ..... | 81 |
| July 12 | 6 | 1.4 | 1.8 | 12.2 | 58 | 84 | 85 |
| Aug. 8 | 7 | 1.0 | 1.1 | ..... | 83 | 86 | 80 |

With barley and Sudan grass seed the percentages which germinated in the different tests show only slight irregularities, with no indication of injury from drying, though the moisture content was reduced below 1 per cent.

With Johnson grass seed there was considerable irregular variation in percentage of germination, probably the result of irregularities in temperature control, moisture content of the germinating bed, and length of germination test, with a slight decrease caused by drying. The last tests, made after 10½ months with the seeds containing only 0.1 to 0.2 per cent of moisture, showed no effect of the drying upon percentage of germination. The seeds which did not germinate in 17 days were tested for viability by removing the scales from the caryopses, breaking the seed covering over the embryo, and then incubating the seeds for an additional period of two or three days. These viability tests showed from 90 to 95 per cent to be alive, both of dried lots and of the control lots.

Wheat samples were first taken six months after the beginning of the test. At that time the wheat contained about 1.5 per cent of moisture

and seemed to show considerable reduction in germinating capacity. However, when the next samples were taken a month later, the dried lots germinated as completely as the control lots, although the moisture had fallen to 1 per cent. The unfavorable results of the previous tests must therefore have been due to causes other than previous desiccation. The wheat was not returned to the desiccators after August 8. Subsequent tests made on September 5 and November 25 gave as complete germination for the dried lots as for the control lots.

The degree of desiccation to which all of the seeds, even of wheat, were subjected without injury, is, of course, greatly in excess of any which occur in nature. Wheat, for instance, when stored under laboratory conditions, contains about 8 per cent of moisture in the winter and much more during humid weather in the summer. Wheat as it comes from the field varies widely in moisture content, but apparently is never below 6 per cent, even in the semiarid regions; the minimum for six years according to figures furnished by the Office of Grain Standardization of the Department of Agriculture, was 6.6 per cent.

## INFLUENCE OF DRYING UPON RAPIDITY OF GERMINATION AND VIGOR OF SEEDLINGS

The germination of the control lots began somewhat more promptly than the germination of the dried lots, but the differences were scarcely perceptible after the second day of the germination test and were probably due in a large measure to an increase in the time required for imbibition before germination could begin.

Table IV gives additional data from the tests begun on September 5, which are typical of data taken from some of the other tests.

TABLE IV.—*Additional data on germination tests begun on September 5, 1917, after seven months' drying*

| Item. | White Smyrna barley. | | | Svanhals barley. | | | Kharkof wheat. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Calcium oxid. | Sulphuric acid. | Control. | Calcium oxid. | Sulphuric acid. | Control. | Calcium oxid. | Sulphuric acid. | Control. |
| Percentage germination in 3 days | 82 | 83 | 83 | 97 | 97 | 97 | 86 | 88 | 90 |
| Percentage germination after third day | 2 | 5 | 3 | 0 | 0 | 1 | 6 | 4 | 2 |
| Number of coleoptiles emerged in 3 days | 6 | 30 | 27 | 14 | 25 | 47 | ..... | ..... | ..... |
| Maximum length of coleoptile on third day.....cm. | 0.2 | 0.9 | 0.8 | 0.2 | 0.8 | 0.7 | 0.4 | 0.4 | 0.6 |
| Average number of roots on third day | 3.2 | 3.4 | 3.0 | 2.8 | 2.9 | 3.0 | 2.1 | 2.3 | 2.5 |
| Maximum length of roots on third day, cm | 5.2 | 4.5 | 4.2 | 4.5 | 5.0 | 4.0 | 2.6 | 2.1 | 3.1 |

TABLE IV.—*Additional data on germination tests begun on September 5, 1917, after seven months' drying*—Continued

| Item. | Pelissier wheat. | | | Sudan grass. | | | Johnson grass. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Calcium oxid. | Sulphuric acid. | Control. | Calcium oxid. | Sulphuric acid. | Control. | Calcium oxid. | Sulphuric acid. | Control. |
| Percentage germination in 3 days | 73 | 76 | 80 | 82 | 88 | 92 | 12 | 19 | 38 |
| Percentage germination after third day | 5 | 4 | 4 | 4 | 4 | 0 | 40 | 41 | 40 |
| Number of coleoptiles emerged in 3 days | ..... | ..... | ..... | 3 | 11 | 157 | ..... | ..... | ..... |
| Maximum length of coleoptile on third day.....cm. | 0.5 | 0.4 | 0.4 | 0.1 | 0.1 | 0.9 | ..... | ..... | ..... |
| Average number of roots on third day | 2.0 | 2.0 | 1.9 | ..... | ..... | ..... | ..... | ..... | ..... |
| Maximum length of roots on third day, cm | 3.2 | 3.5 | 4.2 | 2.1 | 2.0 | 3.0 | ..... | ..... | ..... |

The small number and short length of coleoptiles emerged on the third day from barley samples which had been stored over lime are unusual, as the lots dried over lime did not appear to so poor advantage in any other tests. Except with respect to the development of the coleoptile, little difference appears between the dried lots and the control lots. This difference, however, at least in case of Sudan grass, is not wholly the result of a lower moisture content at the time of beginning the germination tests, as a similar difference appeared in the tests begun on November 25, although with these tests the seeds were left out of the desiccators to absorb water from the air for two days before the germination tests were begun.

The percentages of Johnson grass seeds germinating in three days in case of the tests begun on September 5 showed the effect of the low initial moisture content of the dried lots, but no such difference appeared in the tests begun on November 25 after two days out of the desiccator.

The results outlined in this paper show that all of the seeds used by the present authors, as well as radish seeds as reported by Waggoner, are much more resistant to desiccation than is consistent with Ewart's hypothesis. All of these seeds were dried to 1 per cent of moisture or less without injury; and in the case of Johnson grass seed reduction of the moisture to 0.1 per cent had no injurious effect. Nearly all of the Kentucky bluegrass seeds were still capable of germinating, though with much reduced energy, after the removal of the last trace of water by vacuum desiccation at 100° C. None of the seedlings produced were kept for further growth, but there seems to be no reason to suppose that the dried seeds, except those of bluegrass with the most extreme desiccation, would produce any less vigorous plants than those which were not dried.

SUMMARY

This paper describes experiments to determine the effect on the vitality of certain seeds when dried under varying conditions and for varying lengths of time.

It was found that the percentage of germination was not materially changed when seed of wheat, barley, Sudan grass, Kentucky bluegrass, and Johnson grass was dried to less than 1 per cent of moisture. The percentage of germination of Kentucky bluegrass and Johnson grass seed was not affected when the moisture was further reduced to 0.1 per cent, although the vigor of the Kentucky bluegrass seedlings was greatly reduced. When Kentucky bluegrass seed was further dried in a vacuum oven for six hours at 100° C., the vigor of the seedlings was further reduced, but the percentage of germination was not materially affected. All this controverts Ewart's statements as to the degree of drying which seeds are capable of withstanding and remaining viable, so far as the seeds used in this experiment are concerned.

# OCCURRENCE OF COCCIDIODAL GRANULOMA (OIDIOMYCOSIS) IN CATTLE

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## OBSERVATIONS ON THE DISEASE IN MAN

Wernicke (*12*) [1] in 1892 discovered the parasite of oidiomycosis in a Brazilian soldier suffering from a peculiar skin affection and described it as a protozoon. Later his pupil Posadas (*7*, *8*) made a careful study of the pathologic features of this case, and demonstrated the infectiousness of material from the lesion for several experiment animals. Rixford (*9*, *10*), who in 1894 reported a case in a patient living in California, was the first to describe the disease in this country, and two years later Rixford and Gilchrist (*11*) made a further study of the malady, naming the causative organism "*Coccidioides immitis*," believing it to be of protozoan nature. In 1900 Ophüls and Moffit (*6*), having obtained cultures of the parasite, were the first to class it as a mold, and since that year Ophüls (*5*), Wolbach (*13*), MacNeal and Taylor (*4*), and others have established the exact manner of its development as a parasite and on artificial media.

Coccidioidal granuloma in man does not appear to be a widely distributed affection, nearly all of the cases reported being in patients living in the San Joaquin Valley, California. According to Lipsitz (*3*), out of 40 cases reported up to the year 1916, all but 3 were from this locality, and Dickson (*2*) states that 35 of the patients were residents of California and 3 had visited the State. The relatively small number of cases reported is thought by Ophüls (*5*) to be due to the fact that an occasion for infection is very rarely given; others believe that its striking similarity to tuberculosis and certain other diseases causes even the experienced clinician to err sometimes in exact diagnosis.

The disease is observed most frequently in adult males of the laboring class; the primary infection atria being often found in the skin which has been subjected to injury or pricked by some foreign body, possibly a harborer of the specific fungus. The infection appears also to take place primarily by inhalation and possibly by ingestion. However, there still seems to be some doubt as to the manner of transmission of the

[1] Reference is made by number (italic) to "Literature cited," p. 540.

disease, whether from man to man or whether the parasite passes a stage of its life cycle somewhere in nature and is introduced into the body from this point. Ophüls (5) and MacNeal and Taylor (4) have expressed the suspicion that the disease is an affection of animals and that through association with such as are diseased man may become infected. In view of the writer's positive findings in cattle, these suppositions are well founded.

Practically all the human cases reported have terminated fatally—Wolbach (14) reports a case of recovery—the duration of the disease varying from about three months to nine years. The patients manifest a variety of clinical symptoms which depend upon the organs involved and the extent of the lesions. As the disease progresses from the primary center and becomes generalized, practically all the organs may become the seat of miliary and larger nodules and abscesses, the symptoms corresponding to the location and severity of the lesions. Infection of the bones with purulent osteomyelitis, arthritis, and with compression of the cord and brain is not uncommon. Remittent fever and night sweats appear to be quite constant symptoms, particularly in the later stages of the disease.

## DISCOVERY OF THE DISEASE IN CATTLE AND DESCRIPTION OF THE PARASITE

The present writer encountered the infection in bovine bronchial and mediastinal lymph glands forwarded from an abattoir in San Diego, Cal., by Dr. W. M. MacKellar, of Bureau of Animal Industry. The parasite observed in pus from the glands appears to be identical with that found in the lesions of human cases (Pl. 59, A). In the purulent center of the lesion and in the surrounding granulation tissue the parasite is present in considerable numbers. It appears as a spherical body varying from 3 to 35 $\mu$ in diameter and having a doubly contoured and highly refractive covering, which is from 1 to 5 $\mu$ thick. In some parasites the protoplasm appears very finely granular, while in others it is more coarsely granular and sometimes vacuolated. Large spheres containing many smaller ones of a diameter about 3 to 5 $\mu$ are observed, and occasionally one sees these large bodies in a ruptured state releasing the inclosed spores. The broken empty shells may be seen being invaded by leucocytes. Neither mycelia nor gemmation forms are ever found in the lesions, although the latter type is often simulated when two bodies lie in close contact with each other.

## LIFE CYCLE OF PARASITE

The process of forming mycelia from the spherical bodies was studied by following the method described by MacNeal and Taylor (4). Fresh pus containing spheres was seeded in beef-broth-agar hanging-block preparations and incubated for various lengths of time. After several hours'

incubation microscopic examination of the preparations shows the development of a few short protoplasmic shoots extending out from the capsules of the spherical forms. These growths very shortly assume the character of mycelia, which have a well-defined wall about them, branch extensively, and show septa at intervals. A colony of branched interlacing septate mycelial threads from 2 to 8 $\mu$ in diameter is formed about the capsule of the original sphere in the course of 24 hours. Old cultures, particularly those on potato, show an abundance of aerial hyphæ bearing cylindrical or oval conidia which are surrounded by a doubly contoured membrane with a highly refractive and homogeneous protoplasm (Pl. 59, B).

Wolbach (*13*) and MacNeal and Taylor (*4*) have demonstrated the changes that take place in the development of spheres from the mycelial filaments. When rabbits are inoculated intravenously with masses of filaments and their organs are examined histologically at different stages from 24 hours to several weeks, it may be observed that each sphere develops from a segment of mycelium. At the end of 24 hours most of the filaments have broken up into coarse granules and have largely disappeared; a few, however, remain viable and increase in size, breaking up into rectangular segments which continue to enlarge, so that at the end of seven days perfect spheres have formed, some showing endospores.

MacNeal and Taylor (*4*) have demonstrated *in vitro* on special media and under anaerobic conditions morphologic types, including sporulating forms quite similar to the sperical bodies occurring in the tissues.

## CULTURAL CHARACTERS

AGAR.—The rate of development at room temperature is rather slow, no growth being visible until after three or four days. Incubated at 37° C., colonies are usually visible within 24 hours. These first appear somewhat circular in outline, of a silvery or grayish color, and very slightly raised above the medium. The mycelia penetrate rather deeply into the substratum, giving the colonies so firm an attachment that in removing some of the growth it is necessary to dig into the medium with a strong platinum wire. After several days the culture assumes a whitish moldy appearance caused by the formation of short aerial hyphæ. In some tubes these occur in abundance, attaining a length of 2 to 3 mm. and spread around on the inner wall of the tubes in profusion, while in others they are much less in evidence. In old cultures the medium shows a brownish discoloration, the growth remaining white. As the agar dries out, the growth assumes a slightly yellowish-brown tinge.

GELATIN.—There is a fairly abundant surface growth similar to that on agar. The aerial hyphæ are not usually so plentiful. In about a week or 10 days a slow stratiform liquefaction begins, and eventually the entire mass of medium is liquefied.

POTATO.—The growth is much more luxuriant on this medium than on agar or gelatin, the development of aerial hyphæ being very marked. The medium becomes brownish in old cultures, and the discoloration is imparted to the culture to a certain extent.

EGG MEDIUM.—The growth is somewhat similar to that on potato, except that it occurs around the margin of the medium and extends on to the sides of the tube for the first week or two; later it spreads over the entire surface of the medium. In the

older cultures the medium becomes dark brown, and after 2 or 3 months the growth is discolored.

COAGULATED COW SERUM.—There is a surface membranous growth with little tendency to form aerial hyphæ. Slow liquefaction of the medium occurs after 2 or 3 weeks.

BOUILLON.—The medium is not rendered cloudy, but a fluffy growth appearing like small pieces of cotton develops in the bottom of the tube. In many tubes a rather firm membrane covers the surface of the medium. Aerial hyphæ do not appear except in old cultures.

MILK.—There is a slow digestion of this medium, three or four weeks being required before there is complete clearing. The reaction remains unchanged. A whitish surface membrane is formed.

Indol is not produced. Dextrose, lactose, and saccharose are not fermented with the production of either alcohol or gas.

## THE DISEASE IN CATTLE

Little is known of the disease in cattle resulting from natural infection. The source of infection and the manner of transmission are quite likely the same as in the human cases. However, to judge from the results obtained from experimental inoculations in cattle, these animals are not nearly so susceptible subjects as man.

So far as is known at present, the lesions observed in cattle at the time of slaughter in the abattoir appear to be confined largely to the bronchial and mediastinal lymph glands. These tissues may be the seat of large areas of suppuration or several smaller purulent foci, all of which are usually surrounded by considerable granulation tissue and a fibrous capsule. Upon incising an affected gland there may be squeezed out a thick yellowish and tenacious pus which at once suggests actinomycosis. In fact, the similarity of the lesions produced in the lymph glands by *Coccidioides immitis* and Actinomyces is so striking that the one affection may be easily mistaken for the other upon gross inspection alone. However, microscopic examination of fresh smears of pus at once establishes a diagnosis; in the one case spheres in various stages of development are present in quite large numbers, and in the other the colonies of the ray fungus are detected.

## INOCULATIONS OF EXPERIMENTAL ANIMALS

Successful inoculations were made with guinea pigs, rabbits, dogs, cattle, sheep, and swine, the degree of susceptibility in these animals varying in about the order named. Rapid generalization of the disease usually followed intravenous inoculations, and in the guinea pig and dog subcutaneous inoculation proved fatal in a rather brief period. The lesions most frequently encountered are in the form of miliary or submiliary nodules or abscesses involving practically all the internal organs. The histological structure of the nodules is almost identical with that produced by tubercle bacilli—that is, epithelioid cells and a peripheral zone of lymphocytes with giant cells and central caseation. Inclosed in

most of the giant cells one or more of the parasites can usually be seen. Many parasites are also observed lying free in the tissue.

A considerable number of guinea pigs and rabbits were used in the inoculation tests, but a report on a few typical cases will suffice to show the general character of the lesions produced by the fungus recovered from cattle.

GUINEA PIG 1.—On January 19, 1916, guinea pig 1 was injected subcutaneously with 1 cc. of a suspension in normal salt solution of purulent material taken from bovine glands. After about a week a swelling developed at the point of injection, and later there was ulceration of the skin over this area with the formation of a scab. The animal gradually failed and died on April 1 in an emaciated condition. The autopsy revealed the presence of a local ulcer from which a scanty discharge has escaped, matting the surrounding hairs. This lesion is partially scabbed over, and beneath the scab there is considerable thick yellowish pus in which many spheres are found on microscopic examination. On the floor of the sternum there are two rather large grayish nodules. There are several small nodules in the lungs and spleen. Parasites were demonstrated microscopically in teased preparations from these lesions. Cultures of the mold were obtained from the suprasternal nodule.

GUINES PIG 2.—On April 3, 1916, guinea pig 2 was injected subcutaneously with purulent material from guinea pig 1. It died on June 13. At the autopsy there was observed a local lesion as in the first case; both precrural lymph glands were enlarged and on being sectioned they showed abscess cavities of considerable size containing typical, thick, yellowish pus. On the left side the abscess had broken through the skin. The inguinal, sublumbar, suprasternal, subcostal, and bronchial lymph glands were also involved, all showing suppurating centers of greater or less proportions. Miliary nodules were distributed throughout both spleen and lungs (Pl. 59, C). Parasites were demonstrated microscopically in lesions, and cultures were obtained.

GUINEA PIG 3.—On July 26, 1916, guinea pig 3 was injected intraperitoneally with 1 cc. of a cloudy suspension in a normal salt solution of a 2 month old culture containing many spores. The animal died on September 3. At the autopsy there was observed a rather large abscess in the folds of the great omentum, marked purulent periorchitis, and uniformly distributed miliary nodules in spleen and lungs. Parasites were demonstrated in both fresh and histological preparations. Cultures were obtained.

RABBIT 1.—On July 26, 1916, 1 cc. of material used in the preceding case was injected into the ear vein of rabbit 1. It died on September 17. At the autopsy there are observed miliary foci in lungs, liver, spleen, and kidneys. Similar lesions are found subpleurally and subperitoneally. Parasites were demonstrated in both fresh and histological preparations (Pl. 60, A). Cultures were obtained.

CALVES 177 AND 184.—On April 3, 1916, calves 177 and 184 were injected subcutaneously on the left side of the neck with 4 cc. of normal salt solution suspension of splenic nodules and purulent material from local lesion of guinea pig 1. In the course of a week both animals developed a local swelling about 75 or 100 mm. in diameter. After several weeks a small ulcer was formed from which a slight amount of discharge oozed, gluing together the hairs below the lesion. The ulcer soon scabbed over, and very shortly the skin showed complete healing.

On September 26 calf 184 was killed, and an autopsy was performed. The carcass was in fair condition, and no lesions except the one at the point of injection were found. On being sectioned the local lesion was found to consist of a rather dense layer of fibrous tissue disposed peripherally inclosing a zone of granulation tissue with a purulent center. Many spherical bodies were demonstrated microscopically in fresh

preparations of purulent material from the lesion, and in histological preparations these forms were observed both inclosed in giant cells and lying free in the granulation tissue (Pl. 60, B, C). Cultures were obtained from this case.

On November 9, 1917, calf 177 was killed and a post-mortem examination made. The carcass was in a very well nourished condition. The lesion at the point of injection had almost disappeared, there remaining only a small indurated tumor under the skin which on being sectioned showed a few yellowish foci containing thick purulent matter surrounded by dense fibrous tissue. Parasites were present in the pus. No other lesions were found.

DOG 258.—On October 19, 1916, dog 258 was injected intravenously with 1 cc. of a cloudy suspension of an old agar culture in normal salt solution. In about a week the animal showed symptoms of dyspnea, which rapidly became very much worse, the dog being found dead on October 29. The autopsy revealed the presence of miliary nodules uniformly distributed throughout both lungs; no other lesions were found. Large numbers of spherical bodies were demonstrated in freshly teased preparations of the nodules. Cultures were obtained.

DOG 249.—On October 19, 1916, dog 249 (much larger than dog 258) was injected intravenously with 2 cc. of the above suspension. This animal developed symptoms similar to dog 258, but slighter later, it appearing to show somewhat greater resistance. Death occurred on November 2. At autopsy lesions similar to those in dog 258 were found. Parasites were demonstrated microscopically. Cultures were obtained.

DOG 326.—On October 19, 1916, dog 326 was injected with 2 cc. of above suspension subcutaneously behind its right shoulder. In the course of a week or two a rather extensive swelling developed at the point of inoculation. The hair came off in a considerable area over the swelling, and an ulcer formed in the skin at this point, which after a time scabbed over. The extension of the disease from the primary lesion progressed gradually, the condition of the animal became steadily worse, and death occurred on December 18. At the autopsy extensive ulceration of the skin and deeper tissues was observed at the point of injection. The dependent subcutaneous and intermuscular tissues showed considerable infiltration with inflammatory exudate. Both prescapular glands were enlarged. The lungs, liver, and kidneys were the seat of miliary nodules. There is a nodule about 12 mm. in diameter present in the suprasternal region. A few parasites were demonstrated microscopically in stained sections of the lung nodules.

SHEEP 559.—On October 19, 1916, sheep 559 was injected with 3 cc. of above suspension intravenously. The animal died on June 17, 1917. At the autopsy the carcass was found in a fairly well nourished condition. The superficial tissues in the region of the left shoulder and the right side of the body from the shoulder to the flank showed a rather marked serosanguineous infiltration resulting from injuries inflicted by cattle kept in the same pen. Both submaxillary, both prescapular, both superficial inguinal, the bronchial, and mediastinal lymph glands had small abscess cavities containing yellowish sticky pus. The lungs were the seat of a severe bronchopneumonia with uniformly distributed miliary nodules and larger caseous encapsulated lesions. There was considerable pleuritis, the visceral pleura being greatly thickened with fibro-plastic exudate and adherent in many places to the parietal pleura. There were many miliary nodules in the liver and a few nodules in the kidneys. The parasite was demonstrated microscopically in fresh preparations from lesions in lymph glands, lung, and liver.

SHEEP 560.—On October 19, 1916, sheep 560 was injected with 5 cc. of the above suspension subcutaneously behind its right shoulder. The animal died on July 14, 1917. At the autopsy the carcass was found to be severely bruised; the local lesion, if any was present, being completely obscured by the extensive bruised condition.

The superficial lymph glands appeared free from infection. The lungs showed numerous small caseo-calcareous nodules, well encapsulated, produced by infestation with *Strongylus ovis pulmonaris*. The bronchial and mediastinal lymph glands were normal. The liver, intestines, and mesenteric glands showed severe infestation with *Esophagostoma columbianum*. Microscopic examination of the various tissues failed to reveal the presence of species of Coccidioides.

CALF 176.—On October 19, 1916, calf 176 was injected with 4 cc. of above suspension intravenously. In about a week the animal had marked symptoms of dyspnea, and appetite began to fail. The condition became rapidly worse, and the calf died on November 1. The autopsy revealed the presence of miliary nodules uniformly distributed in both lungs. The bronchial and mediastinal glands were enlarged. No other lesions were present. The parasite was demonstrated microscopically in teased preparations from the lung nodules.

CALF 181.—On October 19, 1916, calf 181 was injected with 5 cc. of the above suspension subcutaneously on the right side of its neck. The local lesion produced in this case was very similar to that noted for calf 177. The animal was killed on November 9, 1917, and a post-mortem examination made. No abnormalities were found, with the exception of the local lesion, which was essentially the same in character as that reported in calf 177. Parasites were demonstrated in pus from this lesion.

PIG 3059.—On October 19, 1916, the right marginal ear vein of pig 3059 was injected with 2 cc. of the above suspension. After about two weeks small warty growths appeared on the surface of the injected ear, showing first in the immediate vicinity of the marginal vein. Somewhat later this warty appearance was observed over a considerable area of the ear which was markedly enlarged and drooping. The general condition of the animal remained very good. This animal was killed on July 17, 1917, and an autopsy made. The injected ear showed a number of small subcutaneous abscesses located chiefly in the region over the marginal vein. The superficial lymph glands were not involved; nor were the bones. The lungs were the seat of miliary nodules. Numerous small nodules were present in the liver, and there were a few in the spleen. The bronchial, mediastinal, and portal glands showed slight lesions. The parasites were demonstrated microscopically in the pus from the ear lesions and in the lung nodules.

PIG 3053.—On October 19, 1916, the right marginal ear vein of pig (sow) 3053 was injected with 2 cc. of the above suspension. Lesions similar to those described for pig 3059 were also noted in this animal. It was observed that for the first few months following the inoculation the ear lesions gradually became worse. Then there was a considerable period in which little change was apparent, and finally there began a recession of the growths. When the animal was killed, on November 9, 1917, no trace of the ear lesions was found at the autopsy. Complete spontaneous healing had taken place. The lungs, however, were the seat of uniformly distributed nodules. These when examined histologically closely resembled tubercle nodules in structure. Parasites were demonstrated in small numbers in the lesions, some appearing to be undergoing degeneration.

On August 10, 1917, this sow farrowed a litter of four pigs, three of which lived, and were kept with the mother until November 9. On February 20, 1918, these three pigs were slaughtered. The autopsies revealed no lesions in any of the three cases.

## ALLERGIC AND SEROLOGICAL TESTS NEGATIVE

With a view to determining whether animals affected with the disease would respond to allergic tests, material for injection was prepared in the following manner: A cloudy suspension in normal salt solution of hyphæ and spores from an old agar culture was autoclaved for 15 minutes

at 15 pounds' pressure and subsequently placed in a shaking apparatus and shaken for three hours. On July 17, 1917, calves 177 and 181 were injected subcutaneously with 5 cc. of this material. The temperatures of the animals prior to injection were normal, and during the next 48 hours neither rise in temperature nor local reaction was noted.

On September 11 the test was repeated, using as injection material 5-cc. doses of a sterilized, filtered, and concentrated (one-tenth original volume) bouillon culture grown for about 6 weeks at 37° C. Negative results were again obtained.

Serums from calves 177 and 181 and from pig 3059 were subjected to both complement fixation and agglutination tests. In the complement-fixation tests two antigens were employed, antigen 1 being the same as the material used in the first allergic test, antigen 2 consisting of some of the substance employed in the second allergic test. No complement-fixing bodies were demonstrated in either case. For agglutination fluid antigen 1 was used. No specific agglutinins were detected.

The negative results obtained in our allergic, complement-fixation, and agglutination tests correspond to those reported by Cooke (1), who states that in a human case no specific complement-fixing bodies or agglutinins could be found in the blood serum, using cultures of *Coccidioides immitis* and emulsions of the same organism from human lesions as antigens. He also states that no specific skin reaction could be demonstrated.

## CONCLUSIONS

(1) Coccidioidal granuloma (oidiomycosis) has been observed in cattle as a natural infection of the bronchial and mediastinal lymph glands.

(2) The disease is transmissible experimentally to guinea pigs, rabbits, dogs, cattle, sheep, and swine.

(3) Cattle affected with this disease show no response to subcautaneous allergic tests.

(4) Neither specific complement-fixing bodies nor agglutinins are detectable in the serums of affected animals.

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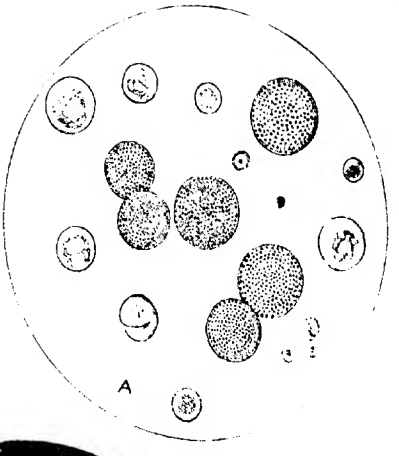
## PLATE 59

*Coccidioides immitis:*

A.—Camera-lucida drawing showing parasites from fresh pus in various stages of development.

B.—Photomicrograph of the hyphæ and spores from an old potato culture. × 220.

C.—Photomicrograph of a nodule of spleen from a guinea pig, showing adult parasites lying free in granulation tissue. × 100.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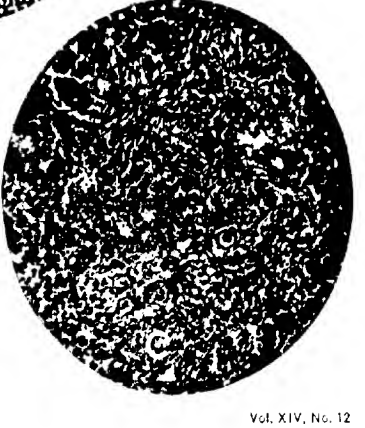
C

# TISSUE INVASION BY PLASMODIOPHORA BRASSICAE

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## INTRODUCTION

Though many workers have studied the clubroot of crucifers, no adequate account has yet been given of the method of infection or of the way in which the parasite becomes distributed in the tissues of its host. The writer has described in detail in a previous publication (*9*)[1] the manner by which a closely related parasite, *Spongospora subterranea* (Wallroth) Johnson, invades the tissues of the potato (*Solanum tuberosum* L.). He has also suggested that a similar kind of infection may hold for *Plasmodiophora brassicae* Wor. and other members of the Plasmodiophoraceae. With this suggestion in mind a study has been made of the clubroot, and it is the chief object of the present paper to give data that seems to make clear the method of tissue invasion.

The occurrence of the parasite within the cells of its host is sufficient proof that it in some way penetrates cell walls. But we would like to know how and when these penetrations take place and the exact method by which the large overgrowth arises. Is the slime mold that produces the great "clubs" with which we are so familiar able to penetrate only the very young rootlets; or is it also capable of attacking older tissues? Does it become distributed by many successive divisions of a few cells originally infected; or is there some other method by which it spreads? Do the many groups of infected cells, the so-called "*Krankheitsherde*," that are distributed throughout the tissues of a single club result from one infection; or is each group the result of a separate infection? What is the relation of the parasite to the host tissues, and by what means does it injure the host plant? These are some of the questions that have not been satisfactorily answered by the students who have already contributed so much to our knowledge of other phases of this interesting disease.

Woronin (*16*) observed amebæ which he believed to belong to *Plasmodiophora brassicae* in the root hairs of young plants and assumed that they would be able to pass deeper into the young root. Favorski (*4*) believes that infection takes place through the ordinary epidermal

[1] Reference is made by number (italic) to "Literature cited," p. 571–572.

cells of very young rootlets rather than through the root hairs. Atkinson (*1*) suggests that the amebæ are capable of—

> streaming out in such fine threads as to enter the roots of the cabbage along with watery solutions of nutrients,

while Eycleshymer (*3*), finding plasmodia in the vessels of fibrovascular bundles, thinks that they may be spread in this manner.

Nawaschin (*13*) observed that infected cells frequently divide and believed that the "*Krankheitsherde*" arise in this way from one or more originally infected cells. He never saw the passage of the parasite from cell to cell and believed that this could not take place after roots begin secondary growth. He says that the tissues probably become infected while very young but that he was unable to observe stages in the process of tissue invasion.

In a recent paper on clubroot Chupp (*2*) takes up the problem of infection and distribution of the organism in the host tissues. Like most other students of the disease, he is of the opinion that only very young rootlets bearing root hairs are susceptible and states that, so far as his observations go—

> there seems to be no question but that penetration does take place through the root hairs and through these only.

He confirms Lutman (*10*) in the observation of actual cell-wall penetrations. In describing the behavior of infection amebæ, Chupp says:

> They may enter in almost a straight path as far as the endodermis,

and argues that a single ameba might give rise to from one to probably six "*Krankheitsherde*" in the primary cortex. He does not describe the infection of the stele, which is obviously the important part of the problem of tissue invasion. Woronin (*16*) long ago pointed out that the primary cortex of young cabbage roots breaks down and is thrown off soon after secondary growth begins. Favorski (*4*) observed that most of the cells infected with Plasmodiophora lie within the central cylinder.

## EXPERIMENTAL METHODS

The methods that have been used in each part of this work will be given at the time the different experiments are described. A few points need mention here. Cabbage plants have been used as the host in all cases. The plants were grown in pots in a greenhouse for winter work. In summer they were grown outside in a garden. Most of the microtome sections were cut in thicknesses varying from 5 to 10 $\mu$. A few have been cut 20 $\mu$ thick. Several fixatives were used to kill material, but Flemming's solutions proved most satisfactory. All of the sections have been stained with Flemming's triple stain. Nawaschin's (*13*) method of leaving sections unbleached so that the oil in the plasmodia, blackened by the osmic acid of the fixative, may serve to make the parasite stand out sharply from the protoplasm of the host, has been used extensively.

## TIME AND NATURE OF INFECTION

It has seemed desirable to study the early stages of infection. This was necessary in order to test the truth of the statement that only very young rootlets are liable to be invaded. We wish to know the exact age at which tissues become immune, whether the parasite can pass through the rootcap and infect the roottip, or whether it passes in through root hairs only. For this work young roots that had been exposed for various periods of time to soil infested with spores of the parasite were fixed in Flemming's weaker solution, embedded in paraffin in the usual way, sectioned on a microtome, and stained with Flemming's triple stain. If the organism enters through root hairs only, it should be possible to find a stage when the root-hair region would be infected and other parts of the young root would still be healthy.

A careful study of many of these sections did not yield the results hoped for. In all cases observed the root was found to be either thoroughly infected or quite free from infection. Stages in the process of infection were not to be observed, and the disease was never confined to any special zone of tissue. One thing that this study did make clear, however, was that the organism sometimes gets into the roottip. Cells in the region of most rapid growth were often found to contain small plasmodia, and the writer was able to confirm the observation made by Nawaschin (*13*) and others that the parasite is distributed to a certain extent by host cell divisions.

Since the stained sections did not show the path of entrance of the organism nor indicate the age of tissue that is attacked, a somewhat different method was resorted to. The soil was carefully washed away from the young roots of some healthy plants. Then a small paper cylinder about 1 cm. long and 0.5 cm. in diameter was slipped over each root. The cylinders were placed at different distances from the roottip and always at a part of the root from which no branches arose. Care was taken not to injure the young roots. They were then filled with moist earth containing spores of *P. brassicae* and the ends were sealed with melted paraffin. After attachment of the cylinders the roots were covered with earth and left for future examination. They were usually examined after a period of approximately two weeks. Infection resulted in practically every instance. That part of the root contained within the cylinder became swollen, no matter whether the cylinder was placed near or far back from the roottip. Those portions of the root outside of the cylinder never showed infection. These experiments proved that the parasite is able to attack tissues far back of the root-hair region, and led to other tests that have yielded interesting results.

It was soon found that the stems of young cabbage plants, as well as the older roots, are susceptible to the disease. The earth was removed from around the stems of plants of different ages growing in pots.

They were then washed clean and a small bit of infectious material was placed on each a short distance below the earth line. Care was always taken to place the infectious material on a portion of the stem that was smooth and free of roots. The inoculum was then sealed to the stem by means of melted paraffin. Sometimes instead of sealing with paraffin it was held in place by wrapping the stem with a small cotton string. Inoculated portions of the stem were wrapped in much the same way that the nurseryman wraps the stems of budded plants. The object was to prevent the inoculum from spreading from the tissue to which it was attached, and both methods served the purpose equally well. After the infectious material was fixed to the stems the earth was replaced and the plants incubated for various periods of time. Plate 61 shows a cabbage plant that was treated in the manner just described. This particular plant was inoculated when about 2 months old. The picture was taken approximately six weeks after inoculation. The small roots coming from the club and from the stem above the club were not present at the time the plant was inoculated. This illustration shows the size of the gall that may result from an original infection of a small circular area of tissue not more than 2 mm. in diameter. It will be seen that only the tissues adjacent to the spot where the infectious material was sealed are diseased. The fibrous roots are all free of disease. Plate 62 shows a portion of two other plants along with the plant shown in Plate 61 for comparison. These two clubs are smoother than that on the plant shown in the middle. This is because fewer branch roots have come from them. The clubs are in general outline spindle-shaped, but they are thicker on one side than on the other. The thick side is the one to which the inoculum was sealed. During the last summer the stems of more than 2,000 plants were inoculated. These plants varied in age from 1 month to more than a year. Without a single exception they became diseased. Old stems an inch or more in diameter became infected almost as readily as young ones.

These experiments bring out two important facts. They show that old tissues are readily penetrated by the parasite and that root hairs are by no means necessary to infection. In the second place they show that the disease spreads from a point of original infection to adjacent tissues.

## MORPHOLOGY OF THE CLUB

The most casual observation of the roots of diseased cabbage plants reveals the fact that many of the overgrowths are not the irregular swellings that one might expect if they resulted from a large number of separate infections by freely moving amebæ each independent of all the others. Such a fortuitous method of infection might give tumors of many different sizes and forms, but it would hardly produce the definite

spindle-shaped clubs that are so characteristic of this disease. If a number of clubs are brought together side by side, it will be seen that although they may differ greatly in size many of them are alike in shape. Some typical spindle-shaped clubs are illustrated in Plate 63. The clubs shown in this illustration were not produced artificially, but were taken from plants grown on an infested field. Those produced by artificial infection of a small circular bit of tissue and shown in Plates 61 and 62 are essentially like the ones resulting from natural infection in the field and shown in Plate 63. The one-sided knobs at X in the figure indicate the point of original infection in each case. Each club is a morphological unit and the result of one primary infection. This fact is very important to an understanding of the disease and lies at the basis of the explanation of the morphological changes which occur.

It often happens, however, that several points that are not very distant from each other become infected. In that case the swellings may fuse together in such a way as to give rise to an irregular-shaped growth or compound spindle. Plate 64, A, shows a portion of two swellings that are about to fuse together. During the later stages of the disease branch roots arising from either the simple or compound spindle become swollen and serve to distort the original form. In this way badly diseased specimens often become quite irregular in shape, but even in these one sees that the overgrowth is made up of a large number of tapering elements. The spindle-shaped tumor so characteristic of the disease results from the reaction of the host to the stimulus produced by the parasite as it spreads gradually through the tissues from the point of original infection.

## STAGES IN CLUB FORMATION

There are two general methods by which cabbage cells become infected. The first may be designated as the direct method and includes all cases of direct penetration. The second is by host cell divisions. This is an indirect method of cell infection. Both of these methods have been known to earlier workers; but the relative importance of the two methods has not been previously recognized. Distribution by host cell division was observed and described by Nawaschin. It will not be taken up in detail in this paper. The direct method of infection deserves further study.

There are two good ways of determining cell-wall penetrations. One is by actual observation of stages in the passage of the parasite through cell walls. The other is by observing the advance of the plasmodia in successive stages of infection. Actual cell-wall penetration will be described later. Advance of the plasmodia in successive stages of infection will be considered here.

The notion that the amebæ of *Plasmodiophora brassicæ* must enter the host through root hairs has gained a strong foothold among students of

the disease. While Favorski (*4*) expressed the opinion that infection takes place through ordinary epidermal cells, he, too, believes that only young roots are susceptible. This mistaken notion has been a great hindrance to a correct understanding of the disease. All previous attempts to study early stages of infection have been carried out with young rootlets. The infection is undoubtedly very rapid in such young organs. The cell walls are thin, and the parasite has to pass through only a few layers of cells before it reaches the central portions of the root. In older tissues the penetration is more difficult; the organism must pass through many layers of cells, and the study of its spread from tissue to tissue is much easier to accomplish. For this reason the writer has used rather old cabbage stems in his study of tissue invasion.

The method has been to pull the earth away from the stems of potted plants, place a bit of inoculum on one side of each stem, and then put the earth back in place. Portions of the stems of these plants were then fixed in Flemming's stronger solution at intervals of one day. This fixing began one day after inoculation and continued for three weeks. At the end of this period infection was evident in the plants first inoculated, for the swellings had reached a considerable size. These stems were embedded in paraffin, sectioned, and stained. None of the cells of any of the stems studied became infected during the first eight days after the inoculum was placed on them. Some of the stems showed a few infected cells on the ninth and tenth days. These cells were in the outermost portions of the secondary cortex. No abnormal growth of infected cells or of cells surrounding them could be observed. On the eleventh day a very small swelling was seen on most of the stems, and somewhat deeper layers of cells showed infection. In a number of cases those swellings were so slight that they could not be seen with the naked eye, and it was not known that the overgrowth had started until after stained sections were studied under the microscope. Plate 64, B, shows a section through a swelling on a stem that was fixed 11 days after inoculation. Only the outer layers of the secondary cortex are infected. The parasite has already stimulated these layers, and they are beginning to show abnormal growth. The dark specks that may be seen in the tissue of the protuberance are the young plasmodia. They are as yet very small, usually showing not more than half a dozen nuclei and little of the oil so characteristically present during the period of their vegetative growth. These small plasmodia gradually increase in size as we pass to later stages of infection. This is well shown in the illustrations. It is interesting to note that the nuclei of the host cells and also the nuclei of the cells immediately surrounding the region of infection are more than twice their normal size. Although the inner cortical tissues are still free of infection, the nuclei of the cells in the cambial region beneath the small plug of infected tissue are much larger than normal cambium nuclei. The

cells in this region are also somewhat abnormally enlarged, but their enlargement has not kept pace with the enlargement of the nuclei. These changes in the cabbage cells in advance of infection show that the growth stimulus acts at a considerable distance from infected cells. This suggests that the stimulus may be some substance which diffuses slowly from infected cells into the surrounding tissues. The cells that contain the parasite are, as might be expected, the ones that make most rapid growth.

The infection continues to spread in all directions during the twelfth and thirteenth days. Plate 65, A, shows a section through a stem 13 days after inoculation. The parasite has passed deeper into the host tissues. Some of the plasmodia have already reached the cambium. Infection has also spread to the sides as well as downward, and the plug of diseased tissue is rapidly becoming larger. Nuclear and cell division as well as cell growth is greatly accelerated. The plasmodia have also increased in size and contain more nuclei and much more oil than they did two days earlier. The parasite seems to be a heavy feeder. Not only does it make rapid growth, but it begins to store up oil very soon after entering the host.

Sections through stems 14 and 15 days after infection show a still further advance of the fungus. It is no longer confined to a small volume of tissue and is spreading rapidly in all directions from the original point of infection. Plate 65, B, shows a section through a stem 15 days after inoculation. Some of the plasmodia have passed beneath the cambium layer. Many of the host cells are very much enlarged, especially near the point of original infection. During the sixteenth and seventeenth days the parasite spreads still farther into the healthy tissues of the stem. It has not penetrated very much deeper, however, and does not seem to be able to attack the woody parts, at least not to any very considerable extent. Plate 66, A, represents a section of a stem 17 days after inoculation. Here the parasite may be seen spreading along the cambium. Plate 66, B, shows a section through a stem 19 days after inoculation. It will be seen that the infected cambium has been active and that growth in this region has contributed very materially to the swelling that is taking place. The outer cortical region has also grown until it is now more than twice as thick as it would normally be. Some of the cells in the cortex are greatly enlarged. Those in the cambial region remain small. While the thickening of the cortex is accomplished more by cell growth than by cell multiplication, the swelling in the cambium region is brought about largely by an increase in the number of cells. Plate 67, A, represents a portion of a section through a swelling on a stem 21 days after inoculation. The infected area is now too large to be included in a photograph of reasonable size. The plasmodia in the tissues of this section are much larger than those found in any of the earlier stages. The parasite has also spread

around the stem and the illustration shows plasmodia in the cambium on the side of the stem opposite the point of original infection.

By this time the disease has passed beyond what may be designated as the early stages of infection. We have seen that the parasite passes successively through the outer layers of the secondary cortex, through the phloem region, into the cambium and finally even into the undifferentiated tissues beneath the cambium. We have also seen that it spreads to the sides as well as downward from the point of original infection, so that by the time the cambium is reached this little plug of diseased tissue has become much broader than it was when infection started. Up to this point the invading organism has followed no special course, but has penetrated with almost equal rapidity in all directions through the bark.

After reaching the cambium the plasmodia no longer penetrate the different tissues with equal readiness, but follow what is undoubtedly the path of least resistance. The intruder now becomes what may be termed a "cambial parasite." Its further spread up and down and around the stem is through the cambium and the layers of undifferentiated cells immediately adjacent. These cells are young and rapidly growing; their cellulose walls are still very thin and probably offer little resistance to penetration. It may also be that in this region of most vigorous growth there is a more abundant supply of food materials than in the older tissues. It spreads through the cambium around the stem until it reaches the side of the stem opposite the point where it originally entered the bark. It also spreads up and down the stem, forming a cylinder of infected cambial tissue. The distance that the fungus travels in its spread through the cambium seems to depend largely on the condition of the host, especially as regards age and rate of growth. If the host plant is young and growing vigorously it may infect the cambium for a considerable distance from the point of original penetration. The writer has observed cases where the cambial infection has extended for as much as 6 inches up the stem from the point where it originally entered. Plate 67, B, shows a longitudinal section through a young stem having a diseased cambium. It should be noted that infection has extended far beyond the region of swelling. The plasmodia are either in the cambium cells or in the cells adjacent to it. The cortex is free of infection for a long distance up the stem. Plate 71, A, shows the distribution of young plasmodia in the cambium of an old stem.

One might think that the disease would continue to spread until the cambium of the entire plant would be infected. This, however, is not the case. At first the spread is very rapid, but during the later stages of the disease it becomes slower and slower and finally almost ceases. Although, as above noted, the swelling does not always extend as far as the cambial infection; this infection, nevertheless, determines very largely the length of the spindle. If the cambial cylinder becomes infected for

a considerable distance from the point of entrance, the spindle will be long; if the infected cylinder is short, the spindle will be short.

The mature spindle or club results largely from the abnormal growth of the infected cambium. By direct penetration of the parasite and through the division of infected cells the disease is spread in both directions from the cambium. If the stem is quite old and the phloem elements well differentiated a large part of the spread from the cambium is through repeated divisions of infected cambial cells or by the division of diseased cells that were split off from the cambium after it became infected. A cross section through such an old stem shows the wood and the outer portions of the bark entirely free of infection. Between this wood and outer bark there is a band of infected tissue. This condition is well shown in Plate 68, A. The inner portions of the wood are entirely free of infection, as are also the outer portions of the bark. For the most part only those tissues that have developed since the cambium became infected show the disease. Plate 68, B, shows a portion of a cross section of a somewhat older stem. It will be seen that the layer of non-infected bark is much thicker here than in A. That the infection shown in B is younger than that shown in A is indicated by the size of the plasmodia in the two sections. The band of infected tissue between wood and bark is very definite in each case. Plate 69, A, gives a portion of a longitudinal section through another stem that became infected after its wood and bark elements were well differentiated. Longitudinal sections through different portions of such a stem show that down near the point of original infection the band of diseased tissue is broad, while farther away it is narrower, and finally comes to a point at the place where the cambium is healthy. The plasmodia shown in Plate 69, A, are almost mature, and spore formation is beginning. The infection here is older than that seen in Plate 68, A, and much older than that shown in Plate 68, B. The three figures show three different stages of the development of the disease in old stem tissues.

If the stem is attacked while the plant is young the greater part of its tissues are still very susceptible to infection The course of infection is much the same in these stems as in the older ones, except that here the fungus spreads more readily from the cambium out into the several layers of the bark and in toward the xylem.

Sections through stems have been used to illustrate the above description of infection. It has been easier to obtain the different stages in the stem tissues than in root tissues. This is probably because the infecting plasmodia travel more slowly through the hard stems than through the roots. It has, nevertheless, been possible to observe many of the different stages of infection in roots also. Although the series of stages is less complete for the root, enough of them have been observed to make certain that the general method of invasion is the same in the two organs.

Two schematic drawings have been prepared for the purpose of indicating in a general way the path followed by the infecting plasmodia and the direction of infection in the different tissues (fig. 1, 2). These drawings apply equally well to both root and stem. The arrows are meant to show schematically the general course taken by the parasite as it passes in through the tissues and produces a typical club. (The actual path along which the infecting plasmodia travel can not, of course, be represented by straight lines.) Besides showing direction of infection, the arrows also indicate the extent to which the different tissues become infected. The cambium is represented by lines without arrow heads. The arrow lines running parallel to it indicate in each case the direction of infection.

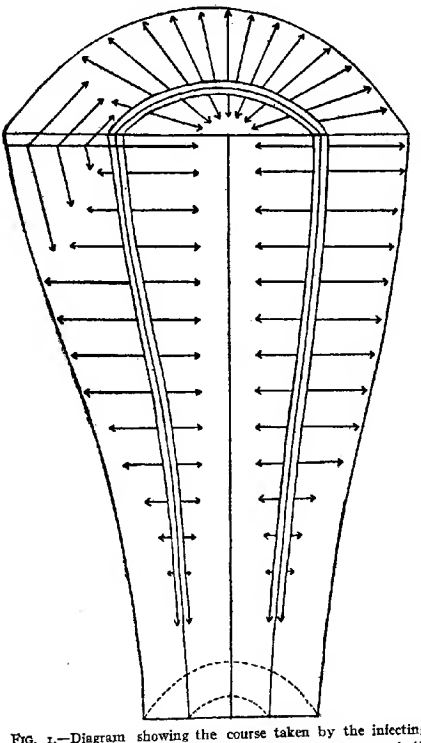

FIG. 1.—Diagram showing the course taken by the infecting plasmodia in a young cabbage root or stem. The arrows indicate the direction and extent of infection.

Each of the two figures represents one half of one end of a club. In order to obtain such a portion the club is cut transversely through its thickest part. Then one of the ends is split longitudinally in half. Figure 1 illustrates the infection of a rather young root or stem, while figure 2 shows the infection of a somewhat older organ. It will be seen that, although the course taken by the parasite in the two cases is the same, the extent of infection is somewhat less in old plants. In the old root or stem the vascular elements are well developed, and the parasite does not penetrate so deep into the tissues on either side of the cambium. In young organs where the vascular elements are not so well developed the plasmodia pass deeper into the tissues on either side of the cambium. The diagrams show the parasite entering the host tissue at the points where the arrow lines pass into the cortex. From this point it spreads to the sides and downward and

finally reaches the cambium. In the cambium it spreads around the stem and also up and down the stem. From the cambium it passes inward toward the pith and outward toward the epidermis. As is shown in the diagrams (fig. 1, 2), most of the cortical tissues become infected from within. This was not to be predicted and is exactly opposite to the direction that previous workers have assumed. The greater part of the cortex becomes diseased through secondary infections rather than by primary infection, as has usually been supposed.

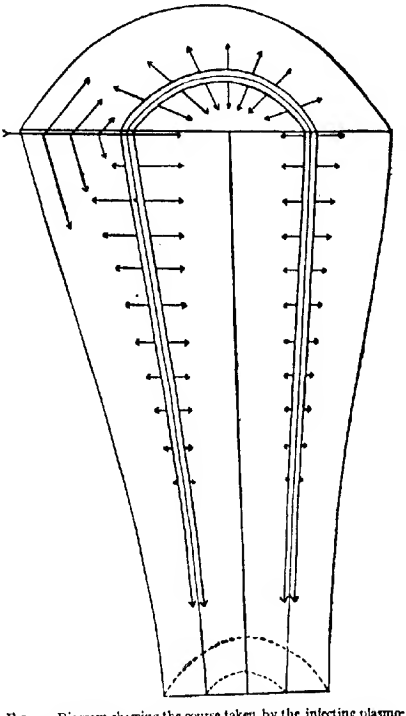

FIG. 2.—Diagram showing the course taken by the infecting plasmodia in cabbage roots or stems that become infected after vascular elements are differentiated. The arrows indicate the direction and extent of infection.

As noted above a few of the outermost cells of the cortex of stems that were fixed nine days after inoculation showed infection. At this time there was no swelling whatever. In no case was an epidermal cell found to be infected. A few young plasmodia were observed in the second, third, and deeper layers of cells. Usually these plasmodia contained from two to six nuclei, but in a few cases uninucleate ameba were seen. In all probability the primary infections are brought about by such amebæ. The writer has never been fortunate enough, however, to observe them passing through the walls of epidermal cells, and until such observations are made it is not possible to say whether uninucleate or multinucleate bodies are concerned. The fact that epidermal cells are seldom infected permanently probably accounts for the difficulty in observing the parasite in these cells. It is also not known whether the disease starts from one primary infection or from the infection of several adjacent epidermal cells. In any case primary infections are local and are confined to very small areas. One might think that, if the spores of *P. brassicae* are closely packed

around the stem of a cabbage plant, infection would be general over the inoculated surface of the stem. This, however, is not the case. Under such circumstances infection starts at a good many different points, but it is never general over the entire surface. No evidence has been obtained that primary infection takes place through wounds. What it is that determines the original point of entrance is a problem that remains to be solved.

## PASSAGE OF PLASMODIA THROUGH HOST TISSUES

As has already been said, both Lutman (*10*) and Chupp (*2*) have described and pictured plasmodia in the act of passing from one cell into another. Neither of these authors has attempted to show the stages by which this process takes place or to describe it in detail. The writer has, therefore, made a further study of this phase of the problem.

In sections of young roots plasmodia were occasionally seen in the act of what seemed to be cell-wall penetration. The process was so seldom observed, however, that the writer was never quite able to convince himself that the appearances met with might not be due to the effect of the fixative on the pathological tissues. In the study of the early stages of infection, cell-wall penetrations were found so frequently as to leave no doubt but that they play a part in normal infection. These penetrations were especially abundant around the edges of the small infection plugs in material that was killed 13 and 14 days after inoculation. Material of this age showed a number of different stages in the process of cell wall penetration. A few instances were observed where plasmodia were closely applied to cell walls but in which no penetration had occurred. Such a case is shown in Plate 70, B. The infected cell is somewhat plasmolyzed by the fixative, but the plasmodium has not been pulled away from the cell wall. The wall, moreover, is seen to bend away from the plasmodium as though a pressure were being exerted on it by the parasite. Plate 70, C, shows another very young plasmodium that has penetrated a cell wall and is beginning to pass through it. This plasmodium contains three nuclei. Figure D shows a little later stage in the passage of a plasmodium through another wall. The parasite has penetrated the wall, but has not yet entered the protoplast of the new host cell. Plasmolysis of the two cells by the fixative has been an advantage in bringing out this fact. The plasmodium contained so much oil that it was not possible to see the nuclei. A still later stage is to be seen in figure E. Here the host cells are not plasmolyzed. The plasmodium is well stained and the opening through which it is passing is clearly shown. Much the same stage is represented in figure F. In this figure the parasite is shown passing through the end of a cell that lies near the cambium. The stage represented in figure G is very interesting because a nucleus is in the act of passing through the opening in the cell wall. This is the only instance in which

such a stage has been found. Here, again, the plasmolysis of the host cells makes the parasite stand out more clearly than it otherwise would. Figure H shows a plasmodium passing through another cell wall. The section in which this plasmodium was found had been left overnight in acid alcohol in order to remove thoroughly the stain from the cell walls. While this was being accomplished, the stain was also removed from the plasmodium to such an extent that its nuclei can no longer be distinguished. Figure I shows a rather large plasmodium in the process of passing through the end of a cell in the region of the cambium.

Late stages in the process of cell-wall penetration have not been found. It may be that these stages require a comparatively short period of time. It may also be that a part of the difficulty lies in distinguishing them from the early stages of penetration because in some cases it is not easy to determine in which direction the plasmodium is passing. The general direction in which migration is taking place helps to determine this point. The bending of the cell wall, where this occurs, is further evidence of the direction of passage. In this way we know that the plasmodium shown in figure I is passing from the cell on the left to the cell on the right. In a number of cases it was observed that the nucleus of the old host cell lies near the plasmodium as it passes out into the new host cell. Figures D, G, and H show such cases. Figure J shows an instance in which the plasmolysis of the host cells seems to have broken a migrating plasmodium into two parts. No hole could be seen in the cell wall, however. A careful study of tissues through which plasmodia are rapidly migrating has been made, but in no case has a hole been found in a cell wall except when a plasmodium is actually passing through the wall. This would seem to indicate that the holes close up soon after the plasmodia pass through. The drawings show that the hole varies considerably in size in the different cases studied.

The migrating plasmodia undoubtedly grow and divide as they pass from cell to cell, but this part of their behavior has not been sufficiently studied up to the present time. In all cases observed the cell out of which the plasmodium is passing is being left free of infection. Such cells act as temporary hosts for the plasmodia that pass through them without leaving any trace of the cells having been infected. While some of the plasmodia move deeper and deeper into the tissues, others remain permanently in certain of the host cells. Here they grow and in time mature spores. The migrating plasmodia are in all cases small. They contain few nuclei and are relatively free of the oil that is so abundant in the larger plasmodia. It is not known what determines whether a plasmodium shall stop in a given cell or shall pass on to some other cell. The writer has observed, however, that the infecting plasmodia in the edges of diseased areas tend to keep a certain distance apart as though they might have a repelling influence on each other. It is not believed

that plasmodia which have grown to be large and are heavily charged with oil are able to leave the cells in which they live. Such plasmodia have never been found penetrating cell walls.

In the above description of cell-wall penetrations the multinucleate masses of parasitic protoplasm have been called "plasmodia." These masses might be looked on by some as multinucleate amebæ. It is usually considered that plasmodia arise through the fusion of many separate amebæ rather than by the growth of a single ameba into a large multinucleate mass. In the saprophytic Myxomycetes this point does not present difficulties because nuclear division in the ameba is closely followed by cell division. In *P. brassicae* nuclear divisions take place without corresponding divisions of the ameboid protoplast. The result is that uninucleate amebæ may grow into large multinucleate masses which can not be distinguished in any way from plasmodia that arise through fusion. For this reason the writer prefers, for the present at least, to designate all multinucleate masses as plasmodia. It should be kept clearly in mind, however, that the term is used here solely for describing the multinucleate masses seen within the cabbage cells, and does not refer to their mode of origin.

As has already been stated, uninucleate protoplasts have occasionally been observed within some of the outer layers of cortical cells. If no swelling has yet occurred on the stem, it is probable that such amebæ have come directly from spores and represent a very early stage of infection. If considerable swelling has already taken place, it is more probable that they have arisen by division from a plasmodium. These uninucleate masses have been observed in cells of the cambium far removed from the point of original infection. They have also been seen in the growing tip of infected stems. Therefore they do not necessarily represent recent infection. Plate 70, K, L, M, shows small ameboid protoplasts that were observed in cells of the cambium of a plant that had been diseased for approximately six weeks. The one is uninucleate, the other binucleate, and the third contains four nuclei. They are the bodies that continue to spread the disease—namely, the infecting amebæ and plasmodia. Plate 70, N, shows a cambium cell containing several small plasmodia. Three of these are binucleate. The host nucleus is shown in process of division. From the distribution of the plasmodia in the host protoplasm it would seem that cell division might leave four of the plasmodia in one of the daughter cells and only one in the other.

## PRODUCTION OF BRANCH ROOTS AND SHOOTS

The distortion of clubs by branch roots has already been mentioned. In fact, the disease seems to stimulate branching. The secondary roots coming from a diseased root may in some instances reach a length of several inches, but they are usually much shorter than this. Sometimes they are so modified that they never get to be anything more than short

knobs. A section through one of these knoblike branch roots is shown in Plate 69, B. When such diseased branches arise from rather young roots, it sometimes happens that the diameter of the branch root is as great or even greater than the diameter of the root from which it arises.

The disease also stimulates the production of buds in places where they do not normally occur. These buds often arise in great numbers from infected roots. This phenomenon was first observed by Woronin (*16*). It has been somewhat more carefully studied by Favorski (4).

The tissues of such buds are always infected. In most cases they grow into short fleshy shoots like that shown on a branch root in Plate 71, A. The leaves are thick, distorted, and abnormally succulent. In other cases the buds grow and give rise to sprouts of considerable size. Such sprouts may push above the surface of the ground and become green. An example is shown in Plate 71, B. The leaves of these large sprouts become green and appear quite normal. Sections through such leaves show that the parasite is more or less evenly distributed throughout their tissues. In some cases there is a tendency for the plasmodia to be more abundant in the tissues bordering on the veins. A cross section through a diseased leaf is given in Plate 72, A.

One interesting thing shown by the diseased buds is that they are often unable to respond normally to gravity. Sometimes they arise on the underside of a root and grow directly downward. In other cases they arise laterally and turn downward like the young sprout shown in Plate 72, B. They have also been observed to grow out horizontally, but this is not so common as downward growth.

The writer has studied the diseased buds with a view of determining whether or not the parasite is distributed in them by the numerous cell divisions that occur in the growing tip. This study has consisted in the observations of serial sections through a few of the diseased buds. The observations have not been extended enough to draw final conclusions, but the indications are that the parasite is not distributed to any great extent by cell divisions in the growing tip. Here, as in old roots and stems, its distribution is by means of direct penetration. The plasmodia follow closely behind the growing tip. Sometimes thay even infect some of the cells of this region. Most of the cells, however, have been found to be free of infection in the cases studied. The distribution of the parasite in these sprouts can not be accounted for by growing tip infection as Favorski (4) seems to have believed.

## HISTOLOGY OF THE CLUB

Some attention should be given here to the response which the host tissues make to the attack of this enemy parasite and more especially to the pathological histology of diseased organs. This phase of the disease has scarcely been mentioned by previous workers. Perhaps the explana-

tion lies in the fact that most investigators have studied only the young rootlets.

As is well known, badly diseased plants wilt during the warmer parts of the day, when transpiration is most rapid. For a certain time at least they recover from the wilting during the night and on cloudy or rainy days. Finally, however, the plants wilt beyond recovery, die, and dry up. The early stages of the disease cause more or less dwarfing, but, so far as the life of the plant is concerned, the critical period is reached when wilting begins. This is the symptom of approaching death.

Most wilt diseases that do not result from an actual destruction of some organ of the plant are caused by vascular parasites. The invading organism gets into the vascular system and interferes with the passage of water from the roots up to the leaves. Several writers have suggested that diseased plants have fewer lateral feeding roots than healthy plants. This is to a certain extent true, but it does not account for the wilting in all cases, since many plants wilt that are abundantly supplied with feeding roots.

The writer has made a study of the vascular elements of infected roots and stems by means of stained serial sections. Small plasmodia have occasionally been found in the tracheids and also in the large vessels. But this is by no means common; it is, in fact, so rare that the possibility of these plasmodia having any appreciable effect on the functioning of the xylem elements is out of the question. Like other dicotyledonous plants, the cabbage during its secondary growth produces, on either side of the cambium, cells that sooner or later become differentiated into xylem and phloem. In the case of the cabbage plant, the differentiation takes place more quickly on the xylem than on the phloem side. The development of xylem tissue thus keeps pace with the increase in transpiration, due to the growth of leaves. When the young root or stem is attacked by the parasite in question the cambium quickly becomes infected, as has been seen. The plasmodia then pass into the layers of undifferentiated cells on either side of the cambium. These cells, instead of developing into vascular elements, as they do in healthy plants, are stimulated to abnormal growth and division. Even the noninfected cells surrounding those that contain the parasite are to a certain degree prevented from developing into vascular tissue. The differentiation of noninfected cells is, however, not entirely prevented, and a small amount of vascular tissue continues to be added to each bundle. The top of the diseased plant keeps on growing—not so fast as in the case of healthy plants—but much too fast for the atrophied development that takes place in the conducting system. In other words, the leaf surface outgrows the conducting system. The parasite may not interfere with the functioning of this system, but it prevents that enlargement which would be necessary to meet the needs of the ever-increasing

number of transpiring cells. The cells of the infected tissues, instead of contributing to water conduction, use up water in their growth. Furthermore, the large swellings themselves, especially when above ground, increase the transpiring surface. Finally on a warm, dry day the critical point is reached. The leaves are no longer able to obtain as much water as they transpire and the plant wilts or, as the gardeners say, "flags."

But not all plants are attacked while still young. It often happens that they escape infection in the seed bed, and contract the disease only after having reached a considerable size. These plants have well-developed vascular systems before they become infected. Can the parasite cause wilting in such plants and, if so, by what means?

In the study of the infection of old stems we have seen that the plasmodia are able to attack the undifferentiated tissues on either side of the cambium, but that it can not penetrate far into the older portions of the bundles. It is unable to attack the woody cells of the xylem, but the undifferentiated cells of the medullary rays are still susceptible. The invader is able to penetrate the rays and to stimulate their cells, so that instead of remaining small and inactive they grow and divide and give rise to a pathological tissue. Whether the medullary cells are penetrated before they begin to grow and divide is not known. The observations of the writer indicate, however, that the growth stimulus travels somewhat in advance of infection.

The growth of the medullary ray cells splits open the woody cylinder. The xylem tissues are forced apart and the bundles distorted in a variety of different ways. Sometimes the splitting up of these tissues is so complete that no two vascular strands can be found near together. In other cases the invasion of the medullary rays is not so complete, and the splitting of the woody cylinder is only partial. It frequently happens that the wood is split into two approximately equal halves.

Plate 73, A, shows a large woody cylinder that is beginning to split apart. The parasite may be seen in some of the cells of the ray that has been stimulated most. Plate 73, B, shows the center of another rather old root. A wedge of diseased tissue has forced apart the two halves. Plate 74, A, shows a further development of the diseased ray. Here the split is complete, and the two halves of the woody cylinder are being forced apart. It will be seen that the right half of the cylinder is beginning to split up into still smaller parts. Plate 74, B, shows a wedge that has grown faster in the center than toward the two edges. It should be noted that the cells composing the wedge are much larger than the cells of the uninfected medullary ray. This is true of noninfected, as well as infected cells. Plate 75, A, shows the woody tissues separated still farther from each other. It is interesting to note that growth is quite uniform in different parts of this diseased ray. The edges of the rays remain almost parallel to each other. Figure B of this

plate shows the halves of another woody cylinder that are being forced farther and farther apart. A longitudinal view of one of these wedges is given in Plate 76, A.

The above examples of ray infection have in most cases shown the wood split into two approximately equal halves. Such cases are easier to show in a photograph than where the splitting is more complete. Plate 77, A, shows a portion of a woody cylinder that is being much more thoroughly split up by medullary infections. By further growth of the infected medullary rays the bundles become more and more separated from each other. Through unevenness in the growth of different portions of diseased tissues they become twisted and distorted in various ways. Sometimes the twisting is so great that a portion of the bundle that was originally nearest the center of the woody cylinder is turned toward the surface of the club, while the portion originally adjacent to the cortex is turned toward the center of the root.

It is interesting to note the further development of the bundles when they are separated from each other in this way. No longer held together in a compact mass, they broaden out so that in cross section they appear fan-shaped rather than wedge-shaped. Figure B of Plate 77 shows a bundle that is beginning to broaden out in this way. Plate 78, A, shows another strand in which the spreading has progressed until the bundle is semicircular in cross section. Figure B of this plate gives a view of a still later development. Here the bundle has grown until it is almost cylindrical and is being further split up by later infections.

The vascular strands, separated from each other and distorted in various ways, are apparently no longer able to function normally. The diseased tissues that separate them probably use up a large part of the water which they transport. In this way the parasite is not only capable of hindering the further development of the conducting system but is also able, by means of medullary infection, to interfere with the functioning of those elements that are already present when it makes its attack. If roots and stems become infected while still young, the injury to their vascular systems consists in hypoplasia of cell differentiation. This also occurs when old organs are attacked, but here, in addition to arresting the development of new xylem and phloem elements, the vascular tissues already present are torn apart through hyperplasia in the medullary rays. For this reason late infection does not prevent the disease from causing the host plant to wilt.

In the above paragraphs emphasis has been placed on the changes wrought by the parasite in the vascular tissues because these changes are so striking and conspicuous. It would be wrong to suppose, however, that these are the only factors concerned in bringing about death or even in producing wilt. The plasmodia in all probability give out substances deleterious to the plant. The yellowing of the leaves during

late stages of the disease suggests the presence of such substances. Moreover, the large galls draw heavily on the food supply of the plant and must lead to profound changes in the metabolic processes going on in the cells of the different tissues.

If roots are attacked while comparatively young, the medullary rays do not for some reason become infected and the woody cylinder remains intact. Most of the abnormal growth that takes place in these roots occurs in the region of the cambium and in the cortex. If, on the other hand, infection takes place after the root is quite old and considerable wood has been produced, the medullary rays, as has been seen, become diseased. The infected rays grow very rapidly, and in many cases give rise to most of the tissues that compose the clubs. The greater part of these tissues are parenchymatous, but weak vascular elements may also develop in them. Such elements are shown in Plates 73, B, and 74, B, and especially in Plate 75, B. They develop in the ray tissues between the bundles and probably aid in transporting water and food to the diseased medullary cells. The long axis of the cells in the tissues between the older portions of the wood are parallel to the direction of growth, while the long axes of the cells between the younger portions of the wood are at right angles to the direction of growth. This is very interesting, since all of these cells arise from the same medullary ray.

The infection of old roots and stems logically falls into four parts, as follows: (1) Infection of the cortex from without (primary infection); (2) infection of the cambium in all directions from the point or points of original penetration; (3) infection of the undifferentiated cells on either side of the cambium and of the inner cortex; and finally (4) infection of the medullary rays. No better proof for direct penetration of tissues by the plasmodia could be wished for than that given by the different stages of medullary infection. It will be seen that direct penetration of the tissues by plasmodia plays a much larger rôle than has previously been supposed.

Former students of the disease seem to have had very hazy notions as to the way in which the galls arise. This is strikingly brought out by Küster's (*8*) reference to gall mother cells. He seems to believe that the cecidia caused by Myxomycetes may result entirely from successive divisions of a single infected cell. We know that individual "*Krankheitsherde*" increase in size through host cell divisions, but these divisions have a small part in distributing the parasite throughout the tissues.

## INFECTION OF YOUNG ROOTS

The writer has not made a detailed study of the infection of very young rootlets. He has no doubt, however, that they become diseased through direct penetration. Even in such young tissues host-cell divisions probably play a minor part in the distribution of the parasite.

Before leaving this subject some mention should be made of the root hair infections observed by Woronin (*16*) and others. The writer has also observed and studied these infections. They are often very abundant and conspicuous. It is not surprising that they should have impressed students of clubroot. The writer has never been able to bring proof, however, that a single one of these infections is caused by *P. brassicae*. In every case where it has been possible to follow the further development of the amebæ and plasmodia observed in the root hairs they have been shown to belong to another organism. Two species of Olpidium are known to infect the root hairs of cabbage plants in Europe. One of these, *Olpidium brassicae*, was observed and described by Woronin (*16*). The other, *O. borzii*, has been studied by Nemec (*14*). The writer has found both of these species on cabbage roots in this country. It is, in fact, difficult to find a cabbage plant entirely free from *O. brassicae*. *O. borzii* is less common, but is by no means rare. Both species have been observed to produce long tubes out of which the zoospores pass.

The writer has grown many cabbage plants in soil free of *P. brassicae*, and has observed in these plants all the different stages of root-hair infection that one finds on the roots of plants grown in infected soil. The living plasmodia in the root hairs closely resemble those of *P. brassicae*. But when killed with Flemming's weaker solution and stained with Flemming's triple stain, they show certain characteristic differences that distinguish them from *P. brassicae*. The distribution of the nuclei in these plasmodia is more regular than in *P. brassicae*, and the structure of the cytoplasm is also different. Plate 76, B, shows a cross section of a young root infected with *Olpidium brassicae*. The dark round bodies are the plasmodia. This parasite gets into the root hairs and into other cells in all parts of the primary cortex. It sometimes occurs sparingly also in the outer cells of the secondary cortex. *P. brassicae* is seldom found in abundance in the cells of the primary cortex or in the outer cells of the secondary cortex. It parasitizes the central cylinder and the inner portions of the secondary cortex. *O. brassicae* is never found in these tissues, so far as the writer has observed. The fields occupied by the two parasites overlap somewhat, but for the most part they are distinct.

There seems to be no good reason why *P. brassicae* should not get into root hairs and other epidermal cells of the primary cortex. No doubt it does pass through these cells on its way to the central cylinder. The writer is forced to conclude, however, that the root hairs are of no importance to *P. brassicae* and that Woronin was in error in believing that the organism he observed in root hairs belonged to this parasite. The same conclusion has already been reached by Favorski (*4*), who made a careful study of the root-hair infections and had at his disposal the slides made by Woronin.

## RELATION OF THE PARASITE TO THE HOST TISSUES

Several previous investigators have given a certain amount of attention to a study of the relation of the parasite to the host cell in which it lives. From this work we know that the host cell is stimulated to abnormal growth and division, that its cytoplasm becomes vacuolate, that its nucleus enlarges and becomes malformed. We also know that this nucleus divides mitotically and that, on the whole, the cell lives and functions more or less normally.

Previous workers seem not to have studied the relation of the parasite to the noninfected cells of the club and to the diseased tissues as a whole. They have looked upon the individual infected cells and groups of cells as separate and distinct pathological units, each independent of all the others. This was, of course, natural so long as it was believed that each diseased cell or group of cells was the result of a separate infection. As soon as it was recognized that the typical club is a pathological and morphological unit and usually the result of a single infection, the question of the relation of the parasite to the tissues as a whole at once presented itself. It has been shown that the organism travels with great readiness through the cabbage tissues. Why is it, then, that some of the cells in each of these tissues always escape infection? Is there any numerical relation between infected and noninfected cells in different clubs and in clubs from different plants?

In order to study these problems, the writer selected at random 60 diseased plants growing in a field near Arlington, Va. The plants were inoculated on July 16 and were taken for study on September 14, just 60 days after inoculation. They were all approximately 3 months old when inoculated and were in a vigorous growing condition. Small portions of tissue were cut from one or more of the clubs of each of the 60 plants. These blocks of tissue were fixed in Flemming's stronger solution, embedded in paraffin, sectioned on a microtome, and stained with the triple stain. They were usually cut so as to obtain a cross section of the club, but longitudinal sections were also made. Sections from each of these plants have been observed under the microscope and have furnished material for a study of the distribution of the parasite in the host tissues.

As might be expected, a good many different stages of the disease were obtained. In some of the clubs the plasmodia were still rather small. In others they were much larger and in still others spore formtion had taken place. No very early stages of the disease were to be found in any of this material. The organism was found to be irregularly but rather evenly distributed throughout the tissues of all the different clubs from all the different plants. Only in rare instances was there to be found large numbers of infected cells adjacent to each other. Many of the cells of every club are free from infection. The diseased

cells are distributed singly or in little groups throughout the matrix of noninfected cells. These groups, the so-called "*Krankheitsherde*," are usually entirely separated from each other by noninfected cells. They may be either large or small, and the cells that compose them may vary greatly in size.

As sections from the different clubs were studied it became increasingly evident that, in spite of the irregularity in the distribution of the parasite, a rather definite relation exists between infected and noninfected cells. If few cells are infected, the plasmodia become large. If many cells are infected, they remain relatively small. Thirty-two of the plants studied showed the fungus in the spore stage. This is the final stage of the disease and, therefore, the one best suited to a study of the relation of the parasite to the tissues as a whole. The observations of sections from each of the 32 different plants showed that the ratio between infected and noninfected cells varies considerably in the different clubs. The relation between the volume of the spore masses and the volume of the host tissues in which they are embedded seemed much more constant. A detailed study has been made of this phase of the relation of parasite to host.

The method used to determine the volume of spores in a given volume of tissue was the following. The sections from each club were observed under the microscope with low-power magnification, and a careful selection was made of a field that seemed to show an average quantity of spores for the tissues of that particular club. The section was then photographed, and a circular picture was obtained of the field chosen. The parts showing spores were then carefully cut out of each photograph. This operation gave many small bits of photographic paper, each showing a picture of one or more spore masses. The remaining portions of the photograph showed only noninfected cells and those parts of infected cells that were free of spores. In this way the photograph was separated into two parts. One part was made up of small bits showing spore masses; the other of small pieces showing cabbage cells only. The two portions were then carefully weighed on a balance and the weights obtained gave the ratio between the area covered by spores and the area showing no spores. It was found that although different sheets of photographic paper vary considerably in thickness, the thickness of different portions of a given sheet is fairly constant. Since the spore masses are shown scattered about over the picture, any slight variation in the thickness of the paper is not a source of much error. The greatest source of error in this method comes from the difficulty of following exactly the outline of the spore masses when cutting them out of the picture with scissors. By careful cutting, this error remains small, and it is believed that the method has given an accurate ratio between the area of the photograph showing spore masses and the area free of spore masses. This ratio has been determined for

each of the 32 plants studied, and the area covered by spores is expressed in Table I as percentage of total area.

Three of the pictures are reproduced in Plates 79, A, B, and 80, A. The spores cover 30.9 per cent of the surface of the photograph shown in Plate 79, A, 28.8 per cent of that shown in Plate 79, B, and 28.8 per cent of that shown in Plate 80, A. It should be noted that the shape and size of the infected cells vary considerably in the three illustrations. Figure B of Plate 79 shows fewer infected cells than either of the other figures. The infected cells are so large, however, that their spore masses occupy a space almost as great as that occupied by the spore masses shown in Plate 79, A. This figure shows a large number of infected cells, but these cells are small and the total spore mass is only slightly greater than in the two other tissues. In spite of differences in the distribution of the parasite, number of cells infected, size and shape of spore masses, and in spite of the differences in size and shape of non-infected cells, each of the three tissues contain approximately the same quantity of spores. The relation between quantity of spores and host tissues was found to hold with remarkable constancy in each of the 32 clubs studied. This is well shown by Table I.

TABLE I.—*Quantity of spores of Plasmodiophora brassicae contained in average sections taken from 32 different cabbage plants*

| Plant No. | Weight of photographic paper— | | Part of photograph occupied by spore masses. | Plant No. | Weight of photographic paper | | Part of photograph occupied by spore masses |
|---|---|---|---|---|---|---|---|
| | Showing spore masses. | Showing no spore masses. | | | Showing spore masses. | Showing no spore masses. | |
| | *Gm.* | *Gm.* | *Per cent* | | *Gm.* | *Gm.* | *Per cent.* |
| 1 | 0.272 | 0.598 | 31.2 | 18 | 0.198 | 0.759 | 20.6 |
| 2 | .236 | .642 | 26.8 | 19 | .256 | .743 | 25.6 |
| 3 | .239 | .645 | 27.0 | 20 | .298 | .665 | 30.9 |
| 4 | .281 | .601 | 31.8 | 21 | .268 | .551 | 32.7 |
| 5 | .271 | .592 | 31.4 | 22 | .286 | .516 | 35.6 |
| 6 | .219 | .619 | 26.1 | 23 | .230 | .607 | 27.4 |
| 7 | .199 | .647 | 23.5 | 24 | .194 | .643 | 23.1 |
| 8 | .180 | .631 | 22.1 | 25 | .250 | .557 | 30.9 |
| 9 | .179 | .663 | 21.2 | 26 | .318 | .520 | 37.9 |
| 10 | .332 | .574 | 36.6 | 27 | .255 | .580 | 30.5 |
| 11 | .220 | .587 | 27.2 | 28 | .206 | .605 | 25.4 |
| 12 | .241 | .615 | 28.1 | 29 | .235 | .612 | 27.7 |
| 13 | .235 | .667 | 26.0 | 30 | .239 | .590 | 28.8 |
| 14 | .256 | .610 | 29.5 | 31 | .175 | .677 | 20.5 |
| 15 | .280 | .690 | 28.8 | 32 | .245 | .675 | 26.6 |
| 16 | .239 | .743 | 24.3 | | | | |
| 17 | .211 | .774 | 21.4 | Average | ..... | ..... | 27.7 |

If the areas shown in the photographs be considered as of unit thickness then the percentages given in the table represent the space occupied by the spores of the parasite in each of the tissues studied. It will be seen that the quantity of spores per unit volume varies somewhat in the

clubs of the different plants. In a few cases the spores occupy only about 20 per cent of the volume of the club, while in other instances they occupy a little more than 35 per cent of the total volume. On the whole, however, the quantity of spores contained in a given volume of diseased tissue is remarkably constant for the different plants studied. The average volume occupied by the spores in the diseased tissues of the different plants amounts to about 28 per cent. This means that approximately 28 unit volumes are occupied by the parasite in every 100 unit volumes of diseased tissue. In other words, the volume relation between parasite protoplasm and host protoplasm may be expressed by the ratio 28 to 72. The writer believes that in this ratio we have expressed numerically the balance which here exists between host and parasite. This balance is not between the individual host cell and the individual plasmodium within it, but between all of the plasmodia and all of the cells of the diseased tissue. The growth of the plasmodia in a given club is determined by the amount of tissue involved. Each club is as thoroughly diseased as is possible. The noninfected cells are apparently free of infection, not because they have accidentally escaped, but because of some influence which the host exercises over the parasite. There is a limit beyond which the parasite can not go in its growth in the cabbage tissues. How this limit is maintained is a problem that remains to be solved. It may be that the spread and growth of the parasite is held in check through the development of some protective substance in the host cells. The infected cells seem to have some means of controlling the growth of the plasmodia which they contain. If we assume that this control is exercised through the production of a protective substance or antitoxin, then it is easy to suppose that this substance might diffuse out into surrounding cells and thus render them immune to attack. Before the parasite would be able to establish itself again in a cell it would be necessary for it to pass beyond the region of immune cells. Such a theory would account for the distribution of the organism and for the balance maintained between it and the host tissues. Acquired immunity has not been observed in plants. Perhaps this is because plants do not have a circulatory system comparable to animals and because the protective substance, if such is developed, is not carried to parts distant from the foci of infection.

Although the relation between quantity of spores and volume of host tissue is very constant, the relation between infected and noninfected cells varies greatly. The fact has been emphasized that many of the cells of most clubs remain free from infection. However, in rare instances, almost all the cells of a club may show infection. It is interesting to note that in such cases the plasmodia in most of the cells remain very small. These plasmodia do not grow and never give rise to large numbers of spores. They also fail to stimulate the host cells to abnormal growth.

An example of this kind of infection is shown in Plate 80, B. Ordinarily the small cells between the groups of enlarged cells are free of the parasite. In this case, however, practically all of these small cells contain small plasmodium. The difference between this and the usual distribution of the disease is strikingly brought out by comparing the two tissues shown in Plate 80. It might seem at first thought that such a tissue as that pictured in figure B would disprove the theory that cells immediately surrounding those that first become infected are rendered immune. The figure shows that these cells are not always immune to attack. But the failure of the plasmodia to develop normally is the best indication of the influence the large plasmodia exert on surrounding cells.

## A COMPARISON OF THE GALLS OF PLASMODIOPHORA BRASSICAE WITH THOSE OF SPONGOSPORA SUBTERRANEA

From the above account of the pathology of *P. brassicae* it will be seen that the disease caused by this parasite differs very materially from that caused by *Spongospora subterranea* (9).

The typical overgrowth caused by *P. brassicae* is spindle-shaped, thick in the middle and tapering gradually toward either end. The overgrowth caused by *Spongospora subterranea* is a knot that protrudes abruptly out of the tissues from which it arises. The nature of the galls produced by these two parasites is in each case the result of a special method of infection. A definite number of cells adjacent to each other are invaded by the plasmodium of *S. subterranea*. The size of the gall produced by this infection depends on (1) the number of cells that are originally infected, and (2) the number of times these cells divide and the size to which they grow. In this way the size of the gall is limited, and it always remains small. It has never been observed that this parasite is able to pass from an infected to a noninfected cell, and there is no indication either direct or indirect that this can occur. It does not spread through the tissues except by means of the large infecting plasmodium. All of the cells of the sorus of *S. subterranea* are infected, and outside of this sorus all of the cells are healthy—that is to say, there is a very definite line between infected and noninfected tissue. If one were to cut out all of the diseased cells in a typical gall of *S. subterranea* he would get only one piece of tissue. This tissue might be called a large "*Krankheitsherde*." On the root and stem of the potato the gall is almost always on one side only; it never becomes a spindle-shaped swelling, but breaks through the epidermis, and exposes the rough surfaces of the infected tissue.

*P. brassicae* behaves very differently in this regard. One or more small bodies of parasitic protoplasm enter the host tissue at some point. These bodies then grow and divide repeatedly. They enter into and pass through the living cells. Some of them become established here and there within the cells of the tissue, while others continue to penetrate

farther and farther into the organ that is being attacked. The period of infection is indefinite; it continues up to the time the host plant dies or stops growing. The infected cells of the gall do not lie adjacent to each other, but are distributed in small groups throughout the diseased tissues. If one were to cut out of a typical gall all of the groups of diseased cells, he would get thousands of distinct and separate pieces of diseased tissue. Instead of one large "*Krankheitsherde*" there are many small ones. The mature gall is seldom or never wholly on one side of root or stem. Usually it surrounds the stem. From the very nature of the infection it is easy to see that *P. brassicae* is a much more dangerous disease than *S. subterranea*. If the writer knew absolutely nothing of the damage done by these two parasites, but had before him the account of the way in which each infects its host tissue, he would be able to predict that the one is a much more serious disease than the other. In the one case the disease spreads indefinitely; in the other it does not. The tumor produced by *P. brassicae* is malignant; that produced by *S. subterranea* is benign.

## OTHER GENERA OF THE PLASMODIOPHORACEAE

We have seen that the method of infection for *P. brassicae* is very different from that found for *S. subterranea*. This raises the question of how other genera of the Plasmodiophoraceae accomplish the infection of host tissues. In order to answer finally the question, it will be necessary to make a careful study of the method of infection for each genus. This the writer has not done. It may, nevertheless, be of interest in the light of what we now know regarding the nature of the galls caused by *P. brassicae* and *S. subterranea* to compare these overgrowths with those caused by some of the parasites in related genera.

SOROSPHAERA VERONICA.—This parasite causes swellings on the aboveground portions of a number of species of Veronica. The galls have been described in detail by Lagerheim and are pictured by Winge (*15*). They are elongated, more or less tapering swellings which resemble in general outline the galls of *P. brassicae*. Stained sections show that these galls are like those of the clubroot in a number of important respects. The outer layers of the cortex are usually free of the parasite. This suggests that infection may proceed from within. The apparent age of the plasmodia in different parts of the tissues also points in the same direction. In one important respect the distribution differs from that of *P. brassicae*. Instead of it being confined to small groups of cells scattered about through noninfected tissues it invades large numbers of cells that are adjacent to each other. On the whole the galls caused by this parasite are so much like those caused by *P. brassicae* that the writer feels justified in predicting that when the method of infection is worked out for *Sorosphaera veronica*, it will be found to resemble closely that described for clubroot.

SORODISCUS CALLITRICHIS.—This parasite produces swellings on *Callitrichis autumnalis* and *C. vernalis*. The galls are round or somewhat elongated. They are usually smaller than those of *Sorosphaera veronica*. The distribution of the casual organism in small groups of cells scattered about in the tissues of the inner cortex (7) shows a close resemblance to the distribution above described for *P. brassicae*. Here, again, infection has spread to all sides of the stem. The round or slightly elongated gall may be looked on as a short spindle, and might be caused by an infection similar to that produced by the clubroot organism, provided the parasite travels very slowly through the tissues.

TETRAMYXA PARASITICA.—Goebel (6) has described and pictured the galls of this parasite on *Ruppia rostellata* Koch. They occur on both stems and leaves and are small and round in shape. It is not possible to determine from his description or his pictures just what tissues are involved. The statement that the region of infection is surrounded by noninfected cortical tissues is very suggestive. What seems to be another species of Tetramyxa has been described by Molliard (12) on *Triglochin palustre* L. This species also parasitizes *T. maritimum* L., and has been studied by Maire and Tison (11). These authors picture cross and longitudinal sections through the galls. The distribution of the parasite in the tissues is strikingly like that of *P. brassicae*. Maire and Tison have described this fungus as a new genus, Molliardia, differing from Tetramyxa in that it does not form spores. But the failure to observe spores is no proof that spores do not occur, and the writer agrees with Winge (15) that, for the present at least, it should be left in the genus Tetramyxa.

OSTENFELDIELLA DIPLANTHERAE.—This parasite which was described and named by Ferdinandsen and Winge (5) causes swellings on the stems of *Diplanthera wrightii* Aschers. The galls produced suggest a method of infection similar to that found for *P. brassicae*. The swelling extends around the stem and the parasite is confined to the cells of the inner cortex. In the upper and youngest part of the overgrowth only uninucleate amebae are present, while in the more mature portions of the gall plasmodia containing many nuclei are found. This suggests that the disease spreads up the stem.

LIGNIERA GRAMINIS.—Two species are included under the genus Ligniera: *Ligniera graminis* (Schwartz) Winge and *Ligniera junci* (Schwartz) Maire and Tison. The genus differs markedly from other genera of the Plasmodiophoraceae in that it does not produce galls. But this failure to stimulate abnormal growth in host tissues is probably connected with the nature of the tissues infected. Only cortical cells are attacked. In all the other genera deeper and more actively growing cells are infected.

From the above discussion it will be seen that the galls produced by *Plasmodiophora brassicae, Sorosphaera veronica, Sorodiscus callitrichis,*

and *Tetramyxa triglochin* are morphologically much alike. In each case the greater portion of the infection is within the central cylinder. The cortex, or at least the outer part of it, is mostly free from disease. The points of similarity suggest that the method of infection may be the same for all of these parasites. The galls caused by *Spongospora subterranea* on the potato differ from those caused by each of the above-mentioned parasites on their respective hosts. This difference is probably correlated with differences in method of host tissue infection.

## SUMMARY

(1) Cabbage plants of all ages up to one year are susceptible to clubroot.

(2) Old plants are almost as susceptible as young ones, provided they are growing.

(3) The typical club is a morphological unit and is usually the result of a single primary infection.

(4) Sometimes the swellings resulting from two or more primary infections may fuse together to produce a compound club. Such clubs are more irregular in outline and often have greater length than those resulting from a single primary infection.

(5) The spread of the disease from points of primary infection is accomplished through direct penetration of cells by infecting plasmodia.

(6) Host cell divisions increase the number of infected cells but have a very small part in distributing the parasite throughout the tissues.

(7) Infection by direct penetration may be divided into four different parts as follows: (1) primary infection of cortical tissues and penetration to the cambium; (2) infection of the cambium in all directions from the point of original penetration; (3) passage of the plasmodia out from the cambium into the cortex and in from the cambium toward the xylem region; and (4) infection of medullary rays.

(8) The infection of a given cell may be either permanent or temporary. If it is temporary, it has no noticeable effect on the cell. If permanent, it stimulates the cell to abnormal growth and division.

(9) The growth stimulus is diffuse—that is, it acts on the noninfected cells of diseased tissues as well as on the infected ones.

(10) The stimulus seems to travel in advance of infection. This is easiest to observe in the early stages of infection and in the infection of medullary rays.

(11) The disease stimulates the production of branch roots and shoots. These branches become infected by direct penetration of plasmodia from the diseased tissues out of which they arise.

(12) Diseased shoots are frequently unable to react normally to gravity, as is shown by their horizontal or downward growth.

(13) A single infection may give rise to many thousands of separate and distinct "*Krankheitsherde.*"

(14) The mass of parasitic protoplasm in a given volume of diseased tissue is remarkably constant in different clubs and in the clubs of different plants.

(15) The average volume relation between host and parasite in the tissues studied is approximately given by the ratio 28 to 72. This is a numerical expression of the balance that exists between host and parasite. A unit volume of diseased tissue always yields approximately the same quantity of spores.

(16) It is suggested that noninfected cells in diseased tissues may be immune.

(17) The wilting of diseased plants is in part due to hypoplasia of cell differentiation in the xylem portions of bundles and to the splitting up of the woody cylinder through the infection and growth of the medullary rays.

(18) The method by which *Plasmodiophora brassicae* infects host tissues differs markedly from that of *Spongospora subterranea*.

(19) If we judge by the kind of galls produced and by the position of diseased tissues it would seem that the method of infection for *Sorosphaera veronica*, *Sorodiscus callitrichis*, and *Tetramyxa palustre* may be similar to that found for *P. brassicae*.

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## PLATE 61

*Plasmodiophora brassicae:*

A cabbage plant 3½ months old; photographed six weeks after being inoculated at a single point on one side of the stem.

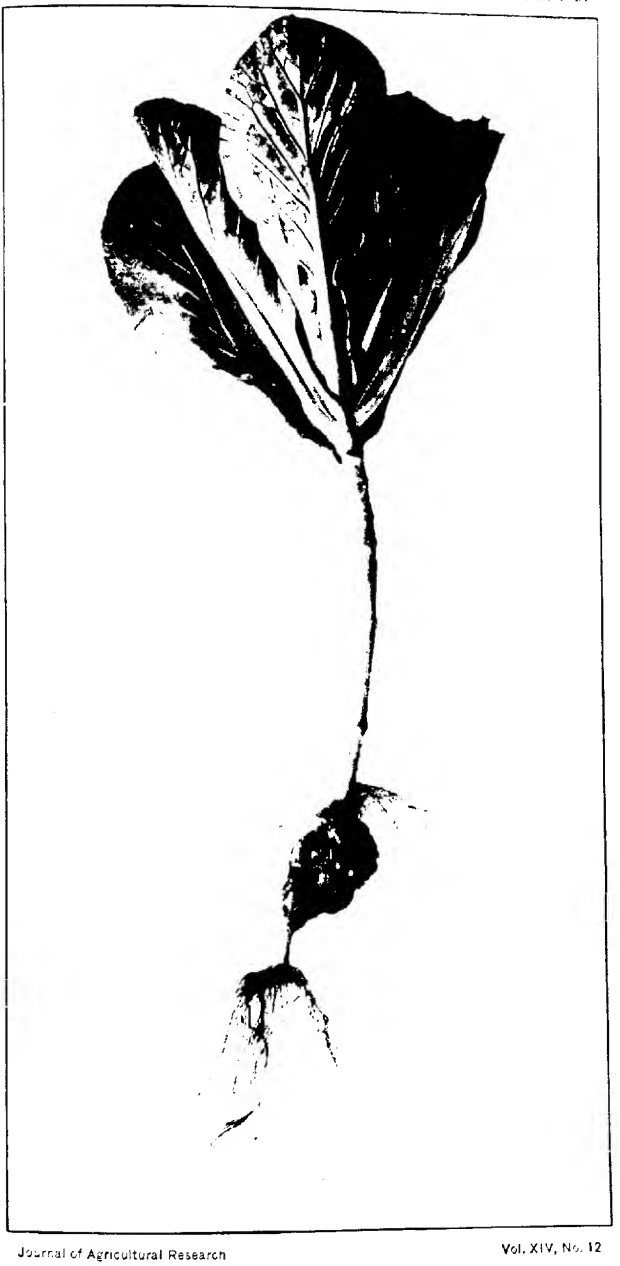


PLATE 62

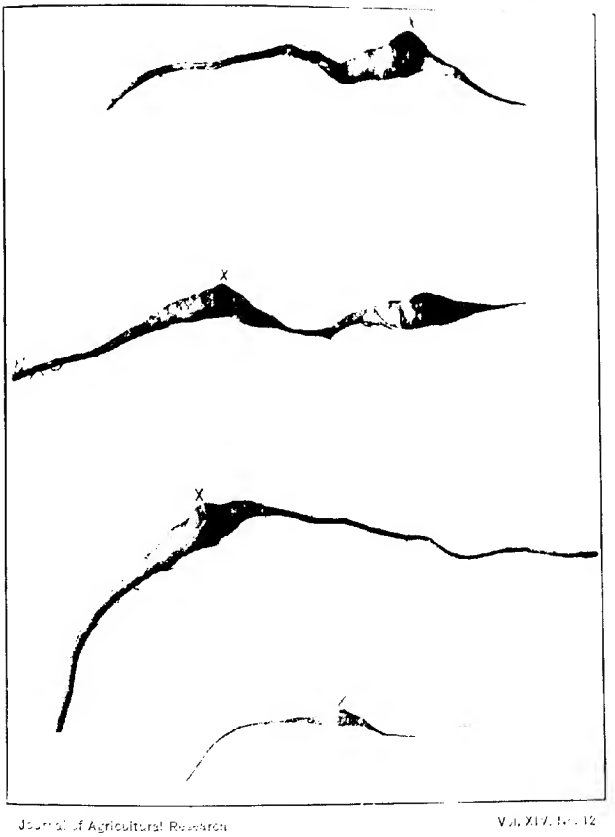
*Plasmodiophora brassicae:*

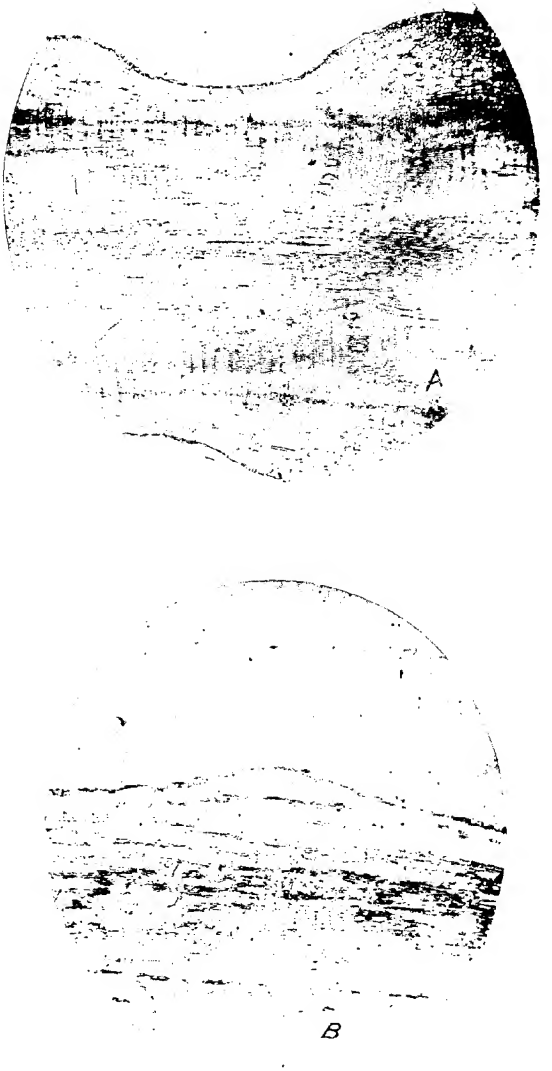
Three clubs, each of which has resulted from the original infection of a small bit of tissue on one side of the stem. They are spindle-shaped and are thickest on the side to which the inoculum was sealed.

PLATE 63

*Plasmodiophora brassicae:*

Some typical spindle-shaped clubs taken from cabbage plants grown in infected soil. The points at which the parasite is believed to have entered the roots are marked by the letter X. Note that the clubs are thickest at these points.


X
X


A
B

## PLATE 64

*Plasmodiophora brassicae.*

A.—A longitudinal section through a young cabbage stem. Infection has taken place at two points on the stem and the swellings are about to fuse together. The cambium between the two swellings is already infected. × 12.

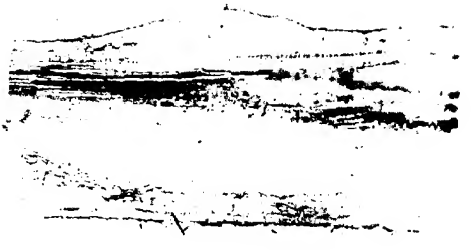
B.—A longitudinal section through a cabbage stem 11 days after inoculation of a single small spot on the stem. The infected tissues are beginning to respond to the parasite. Only the outer portions of the cortex are infected at this time. × 12.

## PLATE 65

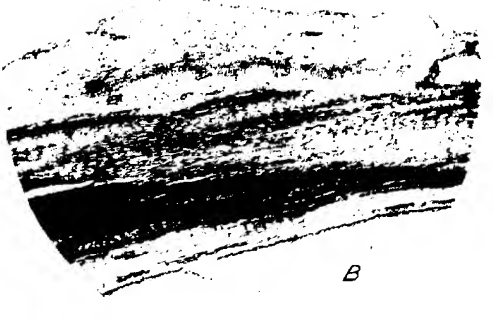
*Plasmodiophora brassicae:*

A.—A section through a cabbage stem 13 days after inoculation. × 12.

B.—A section 15 days after inoculation. Note that the disease has spread deeper into the tissues. In figure A the plasmodia are just beginning to infect the cambium in figure B they may be seen in the tissues beneath the cambium. × 12.

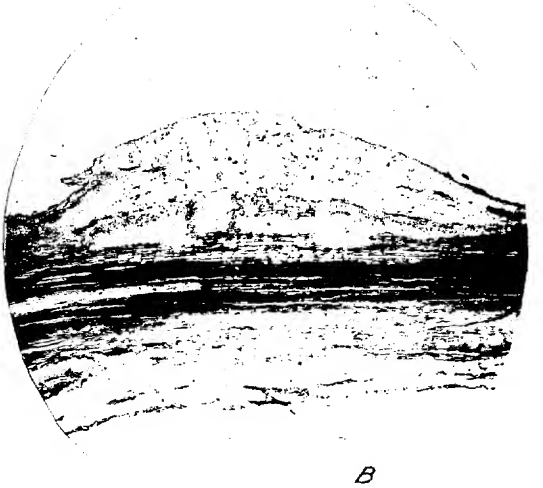


A



B

A



B

## PLATE 66

*Plasmodiophora brassicae:*

A.—A section through a cabbage stem 17 days after inoculation. The swelling is increasing, and the parasite may be seen spreading along the cambium. × 12.

B.—A section through a stem 19 days after inoculation. The swelling is much larger than it was two days earlier. The plasmodia are also getting larger, as is shown by the size of the dark bodies in the cells. × 12.

## PLATE 67

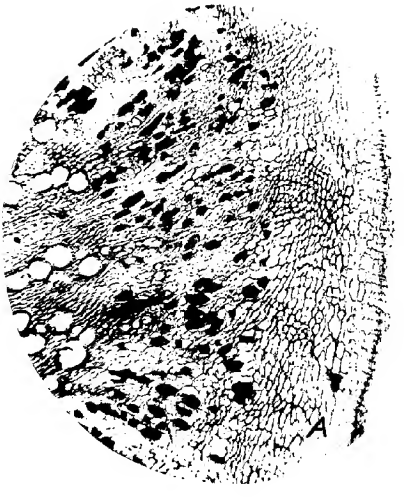
*Plasmodiophora brassicae:*

A.—A section through a cabbage stem 21 days after inoculation. The disease has spread around the stem and the plasmodia may be seen in the cambium opposite the large swelling. × 12.

B.—A stage in the infection of the cambium. The plasmodia have not yet begun to pass out into the cortex or in toward the wood. Note that the cambium is infected far beyond the region of swellings. × 18.


A


B


A


B

PLATE 68

*Plasmodiophora brassicae:*

A-B.—Both figures on this plate show cross sections through cabbage stems that became infected when rather old. The disease is confined to those tissues that were undifferentiated at the time of infection or that have developed since infection took place. × 70.

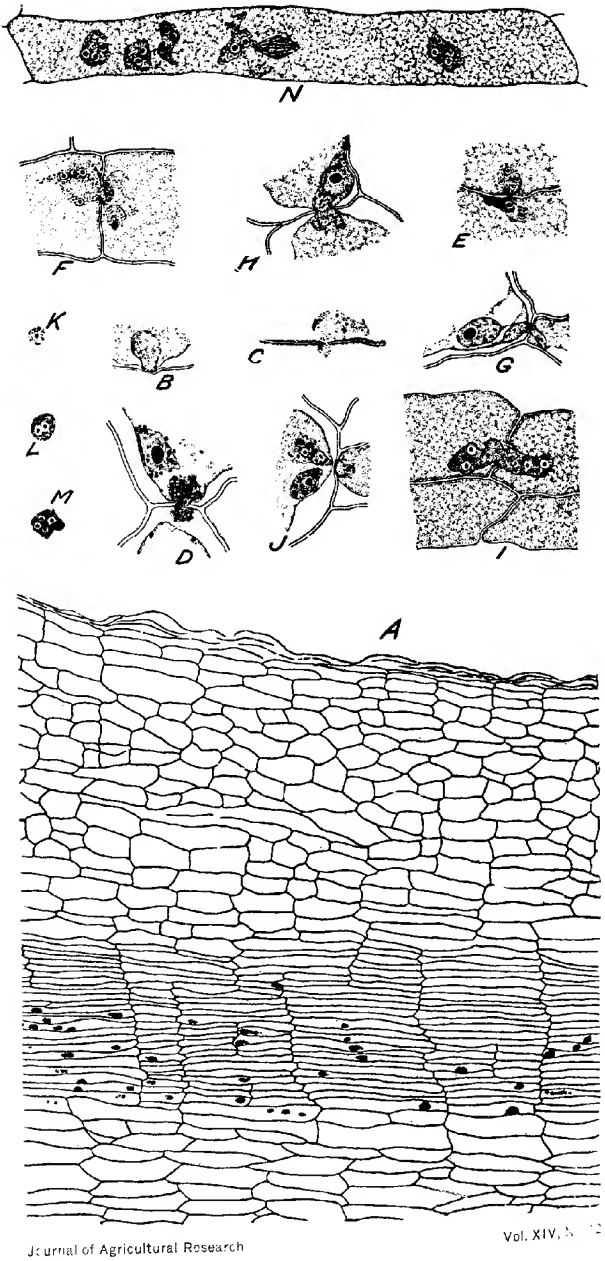
## PLATE 69

*Plasmodiophora brassicae:*

A.—A longitudinal section through a cabbage stem that became infected after the vascular elements were well differentiated. Note that the disease is confined to a definite band of tissue between wood and bark. ×70.

B.—A section through one of the knoblike branch roots that are produced on infected roots. ×60.


A
B


N
F
H
E
K
B
C
G
L
M
D
J
I
A

## PLATE 70

*Plasmodiophora brassicae:*

A.—A portion of a longitudinal section through the stem of a young cabbage plant. The dark bodies represent plasmodia; host-cell contents are not shown. At this stage only the cambial region is infected. ×193.

B.—What is believed to be a very early stage of cell-wall penetration. The plasmodium is closely applied to the cell wall, which seems to be softening and is bending.

C.—An early stage in passage through a cell wall. An opening has been made in the cell wall through which the plasmodium is beginning to pass.

D.—A little later stage than that shown in C. Here the plasmodium has started to pass through the wall, but has not yet penetrated the protoplast of the new host cell.

E, F.—Still later stages in the passage through cell walls.

G.—Interesting because a nucleus is passing through the opening in the wall.

H.—A case in which the opening made in the cell wall is unusually large.

I.—Plasmodium passing through the end of a cell in the region of the cambium.

J.—A case in which plasmolysis of the host cells seems to have broken a migrating plasmodium into two parts.

K.—An ameba taken from a cambium cell.

L, M.—Two small plasmodia that were found in cambium cells far from the point of original penetration.

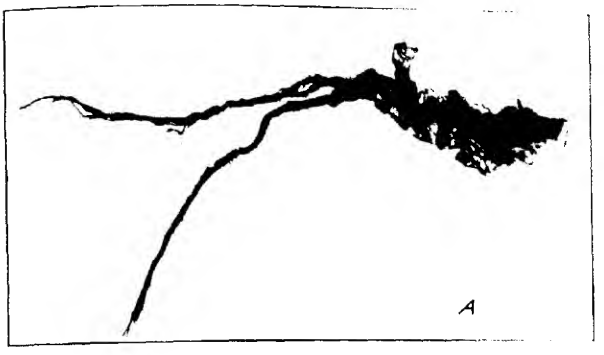
N.—Infected cambium cell. The host nucleus is in process of division. ×836.

## PLATE 71

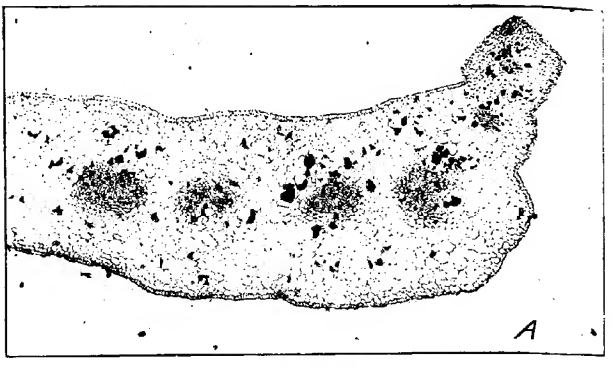
*Plasmodiophora brassicae:*

A.—A young shoot arising from a diseased lateral root of cabbage.

B.—Two large diseased shoots coming from diseased tissue. Here the diseased leaves are quite large and are green.


A


B


A


B

PLATE 72

*Plasmodiophora brassicae:*

A.—A section through a portion of an infected green cabbage leaf. × 40.
B.—An infected shoot that is growing downward.

PLATE 73

*Plasmodiophora brassicae:*

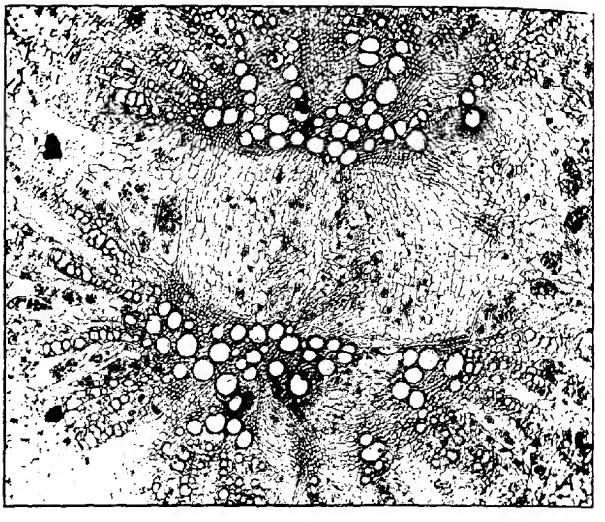
Stages in the infection of medullary rays

A.—A rather large woody cylinder that is beginning to split apart through the abnormal growth of its medullary rays. Several of the rays are being invaded by the parasite. × 40.

B.—The woody cylinder of a cabbage root. It is being split into two almost equal parts by the growth of medullary tissue. × 40.


A


B



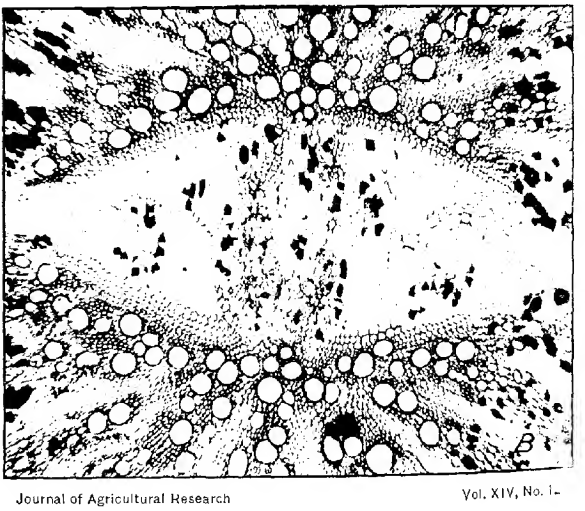


PLATE 74

*Plasmodiophora brassicae*

A.—Somewhat later stages of medullary growth than those shown in Plate 73. The cylinder was first split into two halves. Note that the bundles of the lower half are being still further split up. × 35.5.

B.—Another cylinder being split into two equal halves. This figure shows how the medullary cells increase in size during the first two or three divisions. Two or three rows of cells bordering on the diseased tissue still have the characteristics of medullary ray cells, but the cells of the row adjoining the diseased tissue are much larger than ray cells. × 40.

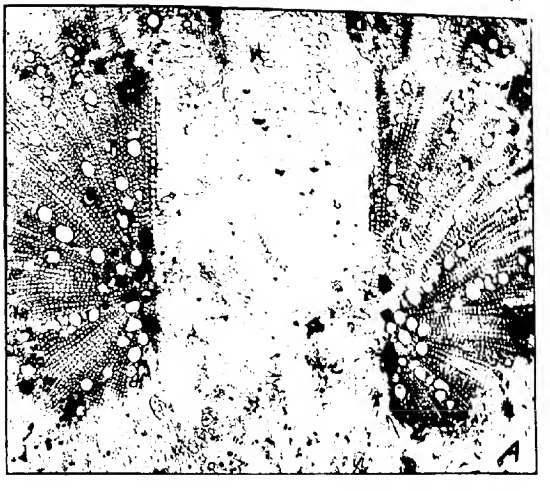
70394°—18——5

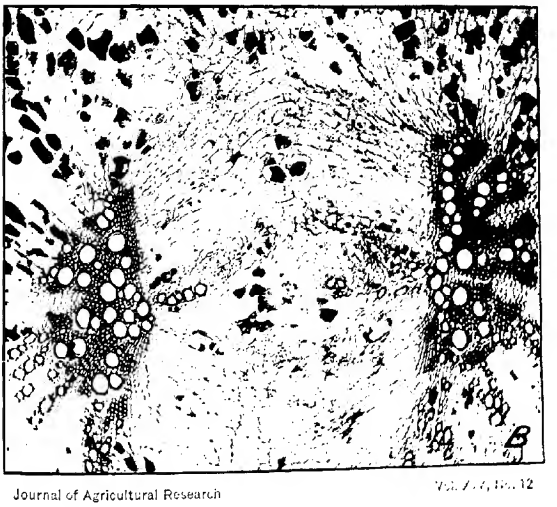
PLATE 75

*Plasmodiophora brassicae:*

A.—The wood of an old cabbage stem that is being split apart by medullary infection. × 40.

B.—A somewhat later stage. Here the two woody halves are being forced still farther apart. × 40.


A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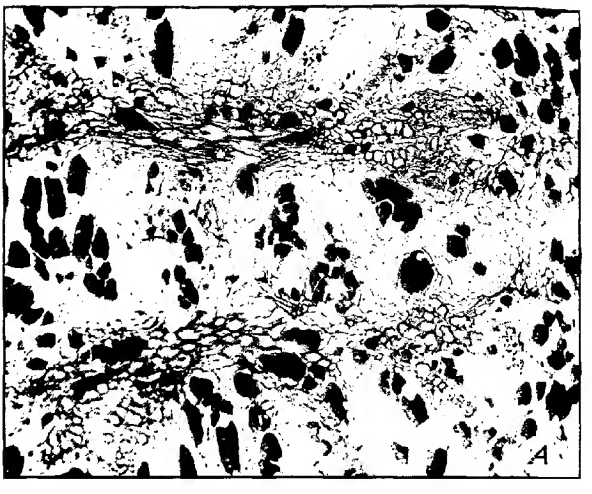
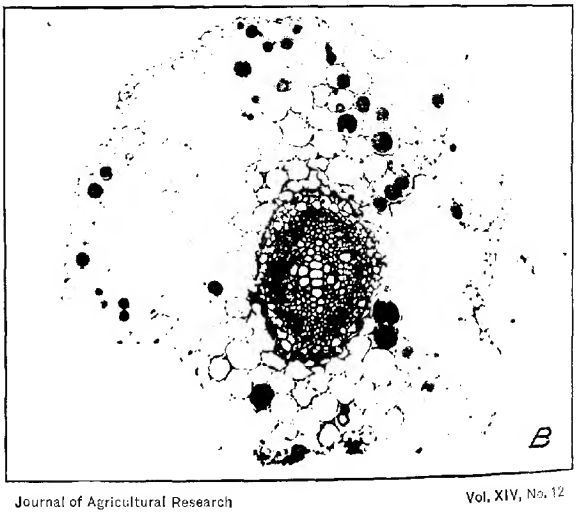

A


B

## PLATE 76

*Plasmodiophora brassicae:*

A.—A longitudinal section through the woody part of a cabbage stem that has been split open by medullary infection. × 70.

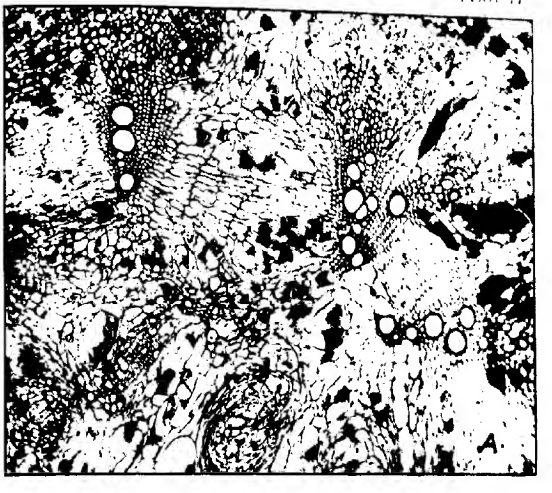
B.—A cross section of a young cabbage root. The ragged outer tissues are the primary cortex. The dark, more or less spherical bodies in the cells of these tissues are the plasmodia of *Olpidium brassicae*. × 80.

PLATE 77

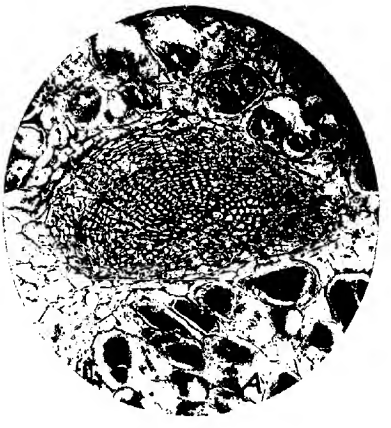
*Plasmodiophora brassicae:*

A.—Several xylem strands being forced apart by medullary infection. × 40.

B.—A bundle that is beginning to be fan-shaped in cross section. This is the result of growth and cell differentiation after the bundle had split off from other bundles. × 40.


A


B


A

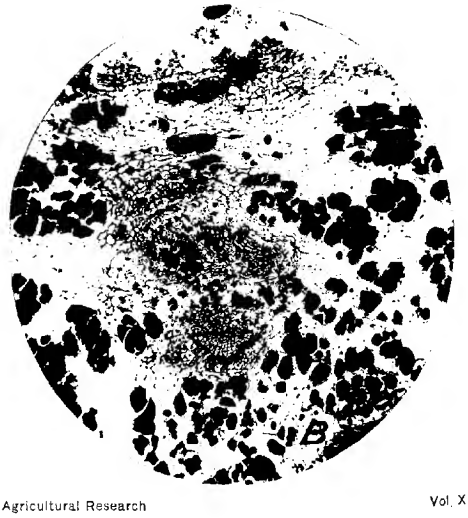

B

PLATE 78

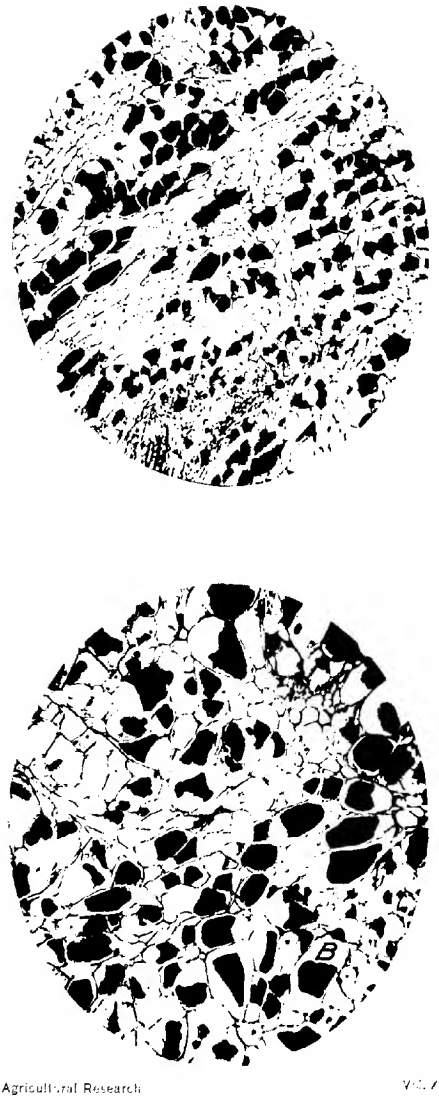
*Plasmodiophora brassicae:*

A.—A fibrovascular bundle that is almost semicircular in cross section. × 100.
B.—A strand that is almost circular in cross section. × 50.

PLATE 79

*Plasmodiophora brassicae:* Distribution of the parasite in the tissues of two different clubs at the time of spore formation—

A.—30.9 per cent of the surface of the photograph is occupied by spore masses.
B.—28.8 per cent of the photograph is occupied by these masses. × 55.


B

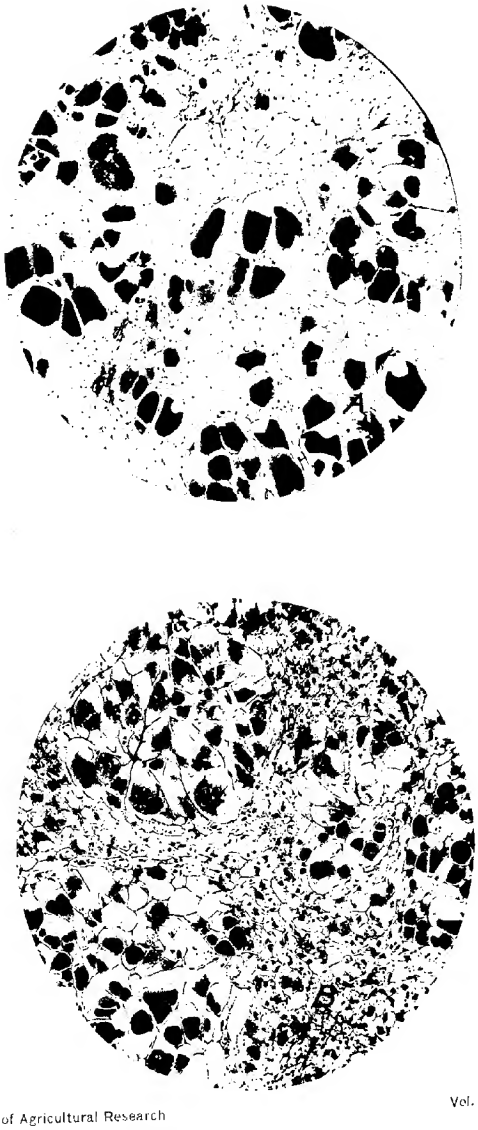


PLATE 80

*Plasmodiophora brassicae*: Tissues from two other clubs.

A.—28.8 per cent of the photograph is occupied by the spore masses. Most of the cells are free of infection. × 55.

B.—An unusual distribution of the parasite. Here practically all cells are infected. × 55.

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